Rhode Island Research Alliance Symposium

"Emerging Biomedical and Life Sciences Research in RI"

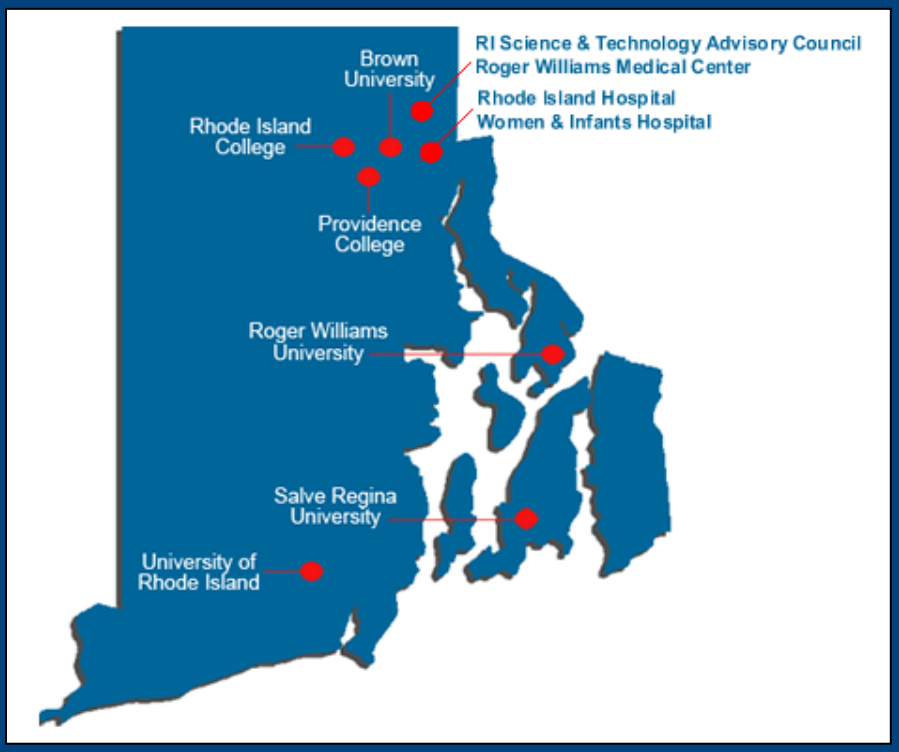
Tuesday, June 3, 2008

8:00 am – 5:30 pm

RI Convention Center

One Sabin Street

Providence, RI



Symposium Sponsors

Rhode Island Science & Technology Advisory Council (STAC)
website: www.stac.ri.gov

Experimental Program to Stimulate Competitive Research (EPSCoR)
Website: www.riepscor.org

COBRE for Perinatal Biology at Women & Infants Hospital
website: http://bms.brown.edu/COBRE

COBRE for Skeletal Health and Repair at Rhode Island Hospital
website: under construction

COBRE for Cancer Research Development at Rhode Island Hospital
website: www.rih-cobre-cares.org

COBRE for New Approaches to Tissue Repair at Roger Williams Medical Center
website: www.rwmc.org/cobre.htm

COBRE for Cancer Signaling Networks at Brown University
website: www.brown.edu/Research/CGP/

IDeA Network of Biomedical Research Excellence at URI
website: www.uri.edu/inbre

Clinical and Translational Science Award, RI – A Planning Grant
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Welcome Letter

RHODE ISLAND SCIENCE & TECHNOLOGY ADVISORY COUNCIL
MAKING INNOVATION CENTRAL TO RHODE ISLAND'S FUTURE

June 3, 2008

Dear Colleague,

Welcome! On behalf of the RI Science and Technology Advisory Council (STAC) and the statewide network of EPSCoR, INBRE, CTSA and COBRE investigators, we are pleased to have you join us today to learn more about the exciting research that is being conducted in core labs throughout our state.

In 2006, to create stronger connections across the state's research organizations and to increase our state's research competitiveness, STAC created the Rhode Island Research Alliance as a platform for promoting collaboration, maximizing state and federal investment in research and enhancing the state's R&D-related economic development opportunities.

Rhode Island is uniquely positioned to leverage its compact geography and densely connected networks to create optimal alignment among its research institutions and organizations, share core resources and expensive equipment, and facilitate face-to-face communication across the state.

By working together, we can maximize state and federal investment in our research programs to make the most of our assets. With collaborative research, everyone wins-- our academic institutions become more competitive for funding, private sector companies get better access to crucial resources and the state enjoys the economic impact of growing our R&D and innovation capacity.

We hope you enjoy the conference and spend some time visiting the poster session, networking with potential collaborators and learning more about emerging biomedical and life sciences research in Rhode Island.

Dr. Clyde Briant
Vice President for Research
Brown University

Dr. Jeff Seemann
Dean, College of the Environment and Life Sciences
University of Rhode Island

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General Information

Registration and Parking
Please stop by the Registration Table between 7:30 – 9:00 a.m. We will have your name badge and the Symposium Program Booklet. The Registration table will be on the 5th Level of the RI Convention Center, in front of ballroom A. Parking is $7 all day if you arrive before 9 a.m., otherwise parking all day will be $8.

Morning Refreshments
Coffee, tea, muffins, bagels, and fresh fruit will be served between 7:30 and 9:00 am in the Rotunda, also on the 5th level.

Morning Session – Invited Speakers
The morning session, starting at 9:00 am, will be held in Ballroom A on the 5th level. There will be a short break around 11:10 am. The morning session ends at 12:45 pm.

Lunch
Box lunches will be available in the Rotunda at 12:45 pm.

Poster Session
The Poster Session will begin in Ballrooms D&E at 12:45 pm. Posters will be available for viewing from 8:00 am to 5:00 pm.

Afternoon Session – Invited Speakers
The afternoon session, starting at 3:00 pm, will be held in Ballroom A on the 5th level. The afternoon session ends at 5:00 pm.

Poster Awards Presentation
At 5:00 pm the Poster Award Presentation will take place in Ballroom A.

Reception
Cocktails and Hors D'Oeuvres will be served from 5:30 pm to 6:30 pm.

PROGRAM

8:00 am Registration in front of Ballroom A - Program Booklets and Name Badges.
Continental breakfast in Rotunda Room, 5th level.

Morning Session, 9:00 am to 12:45 pm

9:00 am ***Ballroom A/5th level – Welcoming Remarks***

NIH Keynote Speaker

9:15 am **Barbara Alving, PhD** - National Center for Research Resources, NIH
"Clinical and Translational Research: Risks and Rewards"
9:45-9:50am 5 minutes Q&A session

9:50 am **Lazaros Kochilas, MD** - COBRE for Perinatal Biology, Women & Infants Hospital
"Inhibition of the Endoplasmic Reticulum Calcium ATP-ase Affects the Early Steps of Left-Right Patterning in Zebrafish Embryos"
10:05–10:10 am 5 minutes Q&A session

10:10 am **Chanika Phornphutkul, MD** - COBRE for Skeletal Health and Repair, Rhode Island Hospital
"Nutrional Regulation of Chondrocyte Growth and Differentiation"
10:25 –10:30 am 5 minutes Q&A session

10:30 am **Arthur Salomon, PhD** - COBRE for Cancer Signaling Networks, Brown University
"Phosphoproteomic Analysis of Cellular Signaling"
10:45–10:50 am 5 minutes Q&A session

10:50 am **Abraham Kovoor, PhD** – INBRE, University of Rhode Island
"Role of RGS9-2 in Schizophrenia and Parkinson's Disease Pharmacotherapy"
11:05-11:10 am 5 minutes Q&A session

11:10 am **BREAK**

11:25 am **Paul Liu, MD, FACS** – Roger Williams Medical Center
"Construction of AAV Vectors for Tissue Repair and Antigenesis"
11:40-11:45 am 5 minutes Q&A session

11:45 am **Timothy Flanigan, MD** - Director, Samuel and Esther Chester Immunology Center, Miriam Hospital
" Clinical and Translational Science Award RI: A Planning Grant"
12:00-12:05 pm 5 minutes Q&A session

12:05 pm **Stephen O'Shea, PhD** – Roger Williams University, EPSCoR
"HPLC/MS methodology for use in profiling fatty acid content in the eggs and larvae and the dermal accumulation of carotenoids in juveniles of the false percula anemonefish *Amphiprion ocellaris"*
12:20-12:25 am 5 minutes Q&A session

12:25 pm **Juan Sanchez-Esteban, MD** – COBRE for Perinatal Biology, Women & Infants Hospital
"Mechanotransduction and Lung Development: Mechanisms and Models"
12:40-12:45 pm 5 minutes Q&A session

PROGRAM continued

12:45 pm **POSTER SESSION AND BOXED LUNCHES**

Afternoon Session, 3:00 pm to 5:00 pm

Scientific Keynote Speaker

3:00 pm **Robert Sackstein, MD, PhD** – Harvard Medical School
"GPS for Stem Cells: The Roadmap for Regenerative Therapeutics"
3:35-3:40 pm 5 minutes Q&A session

3:40 pm **Lei Wei, PhD** – COBRE for Skeletal Heath and Repair, Rhode Island Hospital
" Relocation of HDAC4 regulates Growth Plate Chondrocyte Differentiation "
3:55-4:00 pm 5 minutes Q&A session

4:00 pm **Miran Kim, PhD** - COBRE for Cancer Signaling Networks, Brown University
"Wnt signaling pathway in hepatocellular carcinoma"
4:15–4:20 pm 5 minutes Q&A session

4:20 pm **Steven Symington, PhD** - INBRE, University of Rhode Island
"Pyrethroid modulation of mammalian voltage-sensitive calcium channels"
4:35–4:40 pm 5 minutes Q&A session

4:40 pm **Wolfgang Peti, PhD** – Brown University, EPSCoR
"Structural Basis for Protein Phosphatase 1 Regulation"
4:55-5:00 pm 5 minutes Q&A session

5:00-5:30 pm **Poster Awards Ceremony**

5:30-6:30 pm **Reception**

Keynote Speaker

Barbara Alving, MD, MACP
Director
National Center for Research Resources
National Institutes of Health

Dr. Barbara Alving is the Director of the National Center for Research Resources (NCRR), which funds the development of new technologies for basic and clinical research, supports training for researchers in the biomedical sciences, develops preclinical models, and provides health and biomedical education for the public. The NCRR is responsible for developing the new Clinical and Translational Science Award (CTSA) program that has evolved from the NIH Roadmap initiative to re-engineer clinical research.

Dr. Alving received her M.D. cum laude from Georgetown University School of Medicine in Washington, D.C.. After an internship in internal medicine at Georgetown University Hospital, she completed a residency in internal medicine and a fellowship in hematology at the Johns Hopkins University Hospital in Baltimore, MD. Dr. Alving then became a research investigator in the Division of Blood and Blood Products at the Food and Drug Administration on the NIH campus. In 1980, she joined the Department of Hematology and Vascular Biology at the Walter Reed Army Institute of Research and became Chief of the Department in 1992. She left the Army at the rank of Colonel in 1996 to become the Director of the Medical Oncology/Hematology Section at the Washington Hospital Center in Washington, D.C. In 1999, she joined the National Heart, Lung, and Blood Institute (NHLBI), serving as the Director of the extramural Division of Blood Diseases and Resources until becoming the Deputy Director of the Institute in September 2001. From September 2003 until February 1, 2005, she served as the Acting Director of the NHLBI. From October 2002 until January 06, she served as the Director of the Women's Health Initiative, which is funded through the NHLBI. In March 2005 she became the Acting Director, NCRR and was named Director in April 2007.

Dr. Alving is a Professor of Medicine at the Uniformed Services University of the Health Sciences in Bethesda, a Master in the American College of Physicians, a former member of the subcommittee on Hematology of the American Board of Internal Medicine, and a previous member of the FDA Blood Products Advisory Committee. She is a co-inventor on two patents, has edited three books, and has published more than 100 papers in the area of thrombosis and hemostasis.

Keynote Speaker

Associate Professor, Department(s) of Dermatology and of Medicine, Harvard Medical School
Director of Translational Research, Bone Marrow Transplantation, Massachusetts General Hospital
Physician Active Staff, Dermatology and Medicine, Brigham and Women's Hospital

Robert Sackstein, M.D., Ph.D. received his undergraduate degree from Harvard College, Summa cum Laude in biology, and his M.D. and Ph.D. degrees from Harvard Medical School, where he also received the James Tolbert Shipley Prize for outstanding research. He then completed internal medicine training and fellowships in immunology and hematology at the University of Miami, and received the Young Investigator Award for Excellence in the Field of Hematology from the International Society for Experimental Hematology. Dr. Sackstein is a bone marrow transplant physician and he also performs basic research to find new methods to improve the outcomes for patients undergoing bone marrow transplants. His laboratory efforts have helped define the molecular effectors of lymphocyte migration in graft-versus-host disease, and he is widely recognized for his contributions to the field of glycobiology and for the discovery of HCELL, a glycoform of CD44 that is a potent E-selectin ligand expressed on human hematopoietic stem cells. He is an Associate Professor of Dermatology and of Medicine at Harvard Medical School, and serves as the Head of the Translational Research Program of the Bone Marrow Transplantation Unit at the Massachusetts General Hospital, and is a Bone Marrow Transplant Physician at Brigham and Women's Hospital and the Dana-Farber Cancer Institute.

"GPS for Stem Cells: The Roadmap for Regenerative Therapeutics"

Abstract:
The successful clinical implementation of stem cell-based regenerative therapeutics depends critically on the ability to deliver stem cells to sites where they are needed. CD44 is a transmembrane glycoprotein that is expressed at high levels on most stem/progenitor cells. A specialized glycoform of CD44 called "Hematopoietic Cell E-/L-selectin Ligand" (HCELL) is a potent E-selectin ligand. E-selectin is an endothelial molecule that is expressed constitutively on the luminal surface of bone marrow microvascular endothelium, and is also found on post-capillary venules at all sites of tissue injury. E-selectin receptor/ligand interactions mediate shear-resistant adhesive interactions between cells in blood flow and endothelium, the critical first step in recruitment of circulating cells to any target tissue. We have developed a platform technology called "Glycosyltransferase-Programmed Stereosubstitution" (GPS) for custom-modifying CD44 glycans to create HCELL on the surface of living cells. Ex vivo glycan engineering of CD44 via GPS licenses osteotropism of human MSC to the bone, where these cells differentiate into osteoblasts and produce human osteoid in a NOD/SCID xenotransplant model. GPS technology thus has profound implications in therapy of generalized bone diseases such as osteoporosis. Moreover, because E-selectin expression is universally upregulated on endothelial beds at sites of tissue damage, GPS should enable targeted vascular delivery of stem/progenitor cells for regenerative therapeutics of non-skeletal diseases.

Local Speaker Abstracts

Lazaros Kochilas, MD
COBRE for Perinatal Biology, Women & Infants Hospital
Assistant Professor of Pediatrics, Brown University

"Inhibition of the Endoplasmic Reticulum Calcium ATP-ase Affects the Early Steps of Left-Right Patterning in Zebrafish Embryos"

Problem: Vertebrates develop left-right asymmetries of the heart, brain and digestive system. Calcium signals affect specification of left-right asymmetry, by translating cilia-dependent fluid flow into asymmetric patterns of gene expression. We aimed to determine the role of early calcium signals on the left-right patterning in zebrafish embryos.
Methods: Calcium signals were manipulated in zebrafish embryos using thapsigargin, an inhibitor of the endoplasmic reticulum (ER) calcium ATP-ase pump during early, mid- or late gastrulation as well as early somitic stages. The phenotype was analyzed at the desired stage of development with subtractive imaging, immunolabeling, and mRNA *in situ* hybridization.
Results: Suppression of the ER Ca^{2+} pump elevates cytosolic Ca^{2+} in all embryonic regions and induces randomization of laterality in the heart, brain and gut. Affected hearts displayed either reversed or midline position Brain defects include a left-right reversal of the prominent habenular nucleus and of *pitx2* expression in the dorsal diencephalon. Suppression of the ER Ca^{2+} pump during gastrulation inhibits *no tail* (*ntl*) and *left-right dynein-related-1* (*lrdr1*) expression in the dorsal forerunner cells and affects development of the embryonic organizer (Kupffer's vesicle) in zebrafish. Embryos are most sensitive to inhibition of the ER Ca^{2+} pump during early and mid-gastrulation and lose their sensitivity during early segmentation.
Conclusions: Our data suggest that inhibition of the ER calcium pump by thapsigargin during gastrulation impairs development of the embryonic organizer and disrupts left-right asymmetry. These results suggest an additional role of calcium in left-right asymmetry determination beyond its previously recognized role in sensing fluid flow in the Kupffer's vesicle.

Chanika Phornphutkul, MD
COBRE for Skeletal Health and Repair, Rhode Island Hospital
Assistant Professor of Pediatrics, Brown University

"Nutrional Regulation of Chondrocyte Growth and Differentiation"

Undernutrition is a cause of impaired somatic growth in children. Prior research has focused on the effects of low protein as mediated via the growth hormone/IGF-I axis. Recently, the essential amino acid leucine has been shown to signal via the nutrient sensing kinase, mTOR. Acting through mTOR, leucine can modulate gene transcription, global protein synthesis and mRNA translation. These actions are sensitive to the specific mTOR inhibitor, rapamycin. Further studies have suggested that leucine can regulate translation initiation via a rapamycin insensitive pathway. Our laboratory is focused on the biology of growth plate chondrocytes, the cells that are most critical to linear bone growth. Little is known about effect of leucine deprivation on chondrocyte growth and differentiation. We recently reported the direct effect of mTOR inhibition on chondrogenesis, using the well-characterized ATDC5 chondrogenic cell line and fetal rat metatarsal explants. We demonstrated that rapamycin inhibits the induction of Indian Hedgehog (Ihh) and that Ihh can rescue chondrocytes from rapamycin-induced growth inhibition. Our preliminary data indicate that while lone bone growth is inhibited by both leucine deprivation and rapamycin, mTOR inhibition is incomplete in bones grown in leucine-deprived media, suggesting an alternate, mTOR independent pathway. Microarray data suggested a significant changes in extracellular matrix which may account for the mTOR independent pathway which in turns decreases chondrcyte growth and proliferation. In summary, understanding of the mechanisms by which leucine deprivation inhibits chondrocyte growth and differentiation will enhances our understanding in the effect of malnutrition on long bone growth.

Arthur Salomon, PhD
COBRE for Cancer Signaling Networks, Brown University
Assistant Professor of Molecular and Cell Biology and Biochemistry, Brown University

"Phosphoproteomic Analysis of Cellular Signaling"

Abraham Kovoor PhD
INBRE, University of Rhode Island
Assistant Professor, Biomedical and Pharmaceutical Sciences, University of Rhode Island

"Role of RGS9-2 in Schizophrenia and Parkinson's Disease Pharmacotherapy"

Drug-induced dyskinesia (DID) are unexplained and irreversible movement side-effects of the pharmacotherapy of Parkinson's disease and schizophrenia, and are thought to result from the chronic actions of the respective drugs on striatal D2-like dopamine receptors (D2R). Here we present data that suggest an important role for the striatal specific protein, RGS9-2, in DID. RGS9-2 is a member of the RGS family of Gα GTPase accelerating proteins and RGS9-2 can target to G protein coupled D2R when the two proteins are co-expressed *in vitro*. RGS9-2 and D2R have identical cellular distribution pattern in striatal neurons and D2R abnormally inhibits glutamate-elicited currents in striatal neurons from RGS9 knockout mice. In addition, RGS9 knockout mouse develop drug-induced abnormal movements that more closely resemble DID than in any other rodent model.
From a parallel clinical study we have identifed non-synonymous mutations and intronic deletions in the RGS9 gene that are enriched in patients with schizophrenia and Parkinson's disease and that these RGS9 gene variations produce alterations in RGS9-2 cellular functions.

Paul Liu, MD, FACS
Chairman of Surgery, Associate Professor of Surgery, Boston University School of Medicine, Plastic and Reconstructive Surgeon, Roger Williams Medical Center

"Construction of AAV Vectors for Tissue Repair and Antigenesis"

Tim Flanigan, MD
Director, Division of Infectious Diseases Brown University, Miriam and Rhode Island Hospitals

"Clinical Translational Science Award, Rhode Island: A Planning Grant"

The Clinical and Translational Science Award (CTSA) initiative assists institutions to create an integrated academic home for Clinical and Translational Science that has the resources to train and advance multi- and inter-disciplinary investigators and research teams with access to innovative research tools and information technologies that apply new knowledge and techniques to patient care. Clinical and Translational Science Awards (CTSAs) will attract basic, translational, and clinical investigators, community clinicians, clinical practices, networks, professional societies, and industry to develop new professional interactions, programs, and research projects. Through innovative advanced degree programs, CTSAs will foster a new discipline of Clinical and Translational Science that will be much broader and deeper than their separate components.

Stephen O'Shea, PhD
Associate Professor, Department of Chemistry, Roger Williams University, EPSCoR

"HPLC/MS methodology for use in profiling fatty acid content in the eggs and larvae and the dermal accumulation of carotenoids in juveniles of the false percula anemonefish *Amphiprion ocellaris"*

The successful breeding and growth of tropical marine ornamental fish has been demonstrated to be very closely related to the dietary needs of the brood pair and the healthy development of offspring. Polyunsaturated fatty acids (PUFAS and HUFAS) and carotenoids are important components of marine fish diets and are specifically critical for egg and larval development and the successful rearing of the offspring.

False percula anemonefish (*Amphiprion ocellaris*) breeding pairs were fed each of three different diets for alternating periods of 3 months. Our data indicate that higher fecundity and yield of offspring is a result of both high quality pairs and diets. An improved diet was shown to produce higher quality eggs, but some spawning pairs consistently produce better results than others no matter what diet they are fed. This presentation demonstrates the validation of this new more sensitive and timely efficient method using HPLC/MS technology for the determination of fatty acid profile in developing eggs. Four essential long chain fatty acids were quantified: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosapentaenoic acid (DPA) by HPLC/MS. This method has demonstrated sensitivity of the method over GC/MS limited only by the integrity of the sample oxidation. A followup study was conducted which consisted of feeding the same pairs used in the first study the most productive diet and taking a sample of the eggs produced daily for fatty acid analysis. Metabolic profiling can give an instantaneous snapshot of the fatty acid physiology of the fish egg at a given time after spawning. From these data a direct comparison of egg metabolite physiology (0-9 day development) can be determined to assess the trends for a successful clutch hatch to that of an unsuccessful hatch.

Diets containing high concentrations of carotenoids have been shown in many marine fish to improve vibrancy of external pigmentation and are invaluable metabolic antioxidants. The key color promoting carotenoid in false percula anemonefish, *Amphiprion ocellaris*, is astaxanthin. Juveniles were fed a gel based (Gelly Belly Mix™) diet consisting of 40, 60, 80, or 150 ppm astaxanthin (source Naturose™). Total carotenoid concentration ($\mu g/mm^2$) was quantified spectrophotometrically using the Beer Lambert Law (λ = 480 nm and $E^{1\%}_{1cm}$ = 2100) and ImageJ. Results were correlated to visual vibrancy (%Mean-Red-Pixels (%MRP)) obtained utilizing Photoshop CS2. Results showed no significant difference in total carotenoid accumulation rate across treatments. Significant increase in total concentration as a function of time was observed. Color analysis showed significant differences in %MRP values even though treatments did not produce significantly different carotenoid accumulation rates.

Juan Sanchez Esteban, MD
COBRE for Perinatal Biology, Women & Infants Hospital
Assistant Professor of Pediatrics, Brown University

"Mechanotransduction and Lung Development: Mechanisms and Models"

Mechanical forces are critical for normal lung development. However, the mechanisms by which pulmonary cells sense and transduce mechanical signals are largely unknown. Using an *in vitro* experimental system simulating mechanical forces in lung development, we have previously found that several receptors and signaling pathways are stimulated by stretch. In this talk, we will present new findings demonstrating how mechanical stretch promotes differentiation of fetal type II epithelial cells via ectodomain shedding of EGFR ligands. We will also describe a mechanistic model on how this shedding process might be mediated. Finally, we will discuss organotypic models of mechanical stretch in fetal lung development.

Lei Wei, PhD
COBRE for Skeletal Health and Repait, Rhode Island Hospital
Assistant Professor of Orthopaedics, Brown University

"Relocation of HDAC4 regulates Growth Plate Chondrocyte Differentiation"

HDAC4 is a negative regulator of growth plate maturation that works by binding to and inhibiting the activity of Runx 2/Cbfa1 in the nucleus. However, the mechanism is unknown. We hypothesize HDAC4 nuclear-cytoplasmic shuttling controls chondrocyte differentiation and is dependent on the Ca^{2+}/calmodulin signaling pathway. Our studies demonstrate HDAC4 is located in the nuclear at the proliferation zone while HDAC4 is relocated to cytoplasmic at pre-hypertrophic zone in the growth plate in vivo. We further found that CaMKIV regulates the nuclear-cytoplasmic shuttling of HDAC4. Active CaMKIV increases the HDAC4 relocation from nuclear to cytoplasm and binds to 14-3-3 protein while the inactive CaMKIV does not induce HDAC4 relocation and binds to 14-3-3 protein. RT-PCR indicates that the relocation of HDAC4 regulated by CaMKIV induces an increase in Ihh and type X collagen expression; they are markers for pre-hypertrophic and hypertrophic chondrocytes respectively. The data indicates the relocation of HDAC4 controls proliferative chondrocyte differentiation into prehypertrophic chondrocytes in growth plate.

Miran Kim, PhD
COBRE for Cancer Signaling Networks, Brown University
Assistant Professor of Medicine, Brown University

"Wnt signaling pathway in hepatocellular carcinoma"

Hepatocellular carcinoma is one of the most prevalent malignant neoplasms worldwide. Although the major etiologies of HCC are now well defined, the molecular mechanisms that contribute tumor initiation or progression of HCC are poorly understood. There is increasing evidence that aberrantly activated the canonical Wnt signaling is a common event in the pathogenesis of HCC. We aimed to define the molecular mechanisms of liver oncogenesis via Wnt signaling pathway. In human HCC samples, the expression level of Wnt3 (an activator for canonical signaling) was upregulated in HCC tumors compared to adjacent uninvolved liver. On the contrary, the level of Wnt11 (an activator for non-canonical) was downregulated in tumor tissues. We also found that Wnt11 antagonized the canonical signaling in HCC. Moreover, the biological function of Wnt11 opposes the activity of Wnt3, which stimulates HCC cell proliferation and motility. It appears that Wnt3 and Wnt11 may act in concert to maintain hepatic canonical Wnt homeostasis, and subsequent alterations in the balance may contribute to the development of HCC. Since dys-regulation of Wnt/b-catenin signaling has been shown to be important during tumor formation, further analysis of these two signaling pathways may provide attractive molecular targets for therapy of this disease.

Steven B. Symington, PhD
INBRE, University of Rhode Island
Assistant Professor, Department of Biology and Biomedical Sciences
Salve Regina University

”Pyrethroid modulation of mammalian voltage-sensitive calcium channels”

Problem: Pyrethroids are widely used insecticides in both agricultural and vector control programs. Given the widespread use of pyrethroids for the control of insect vectors of devastating human and animal diseases (particularly in urban environments) and additional exposure via dietary uptake, human consumption is virtually assured. The purpose of this research was to determine the effects of pyrethroid insecticides on the current characteristics of a human t-type voltage-sensitive calcium channel ($Ca_v3.2$).
Methods: Human $Ca_v3.2$ cDNA was transcribed into cRNA using the mMessage mMachine *in vitro* transcription kit and injected into defollicated *Xenopus* oocytes. Human $Ca_V3.2$ currents were electrophysiologically characterized using the two-electrode voltage clamp technique with Ba^{2+} as a charge carrier. Pyrethroids effects on the overall peak current, voltage-dependent activation and inactivation, and steady-state activation and inactivation tau were determined following perfusion with various concentrations of deltamethrin and enantiomers of cypermethrin.
Results: Deltamethrin reduced peak current in a concentration-dependent manner with an approximate EC_{50} of 10^{-13} M. Furthermore, 40% of $Ca_v3.2$ peak current was inhibited by 10^{-7} M deltamethrin, a concentration that elicits a maximum response, compared to $Ca_v3.2$ expressing oocytes treated with DMSO (solvent control). In other preliminary experiments, 1R-cypermethrin (a toxic enantiomer) inhibited peak current in a similar fashion as deltamethrin. The non-toxic, 1S-cypermethrin had no effect on $Ca_v3.2$ peak current compared to DMSO control.
Conclusions: Pyrethroids are potent and stereospecific inhibitors of the human t-type voltage-sensitive calcium channel ($Ca_v3.2$)

Leslie Cousens, PhD
Research Assistant Professor of Medicine, Boston University School of Medicine
COBRE for New Approaches to Tissue Repair, Roger Williams Medical Center

“Development of Novel Bispecific Antibodies for Facilitating Site-specific Tissue Repair”

Bispecific antibody technology has the potential to combine effector cells that may facilitate the repair and regenerative processes with an injury-specific targeting antibody to create a biologic “drug” for the purpose of enhancing wound repair. We hypothesize that bispecific antibodies will direct trafficking of specific cell populations, more or less differentiated, to sites of injury where they can facilitate tissue repair and regeneration.
To test this hypothesis, we will combine (chemically heteroconjugate) one mAb specific for a target cell population (*i.e.* F4/80 expressed by macrophages, c-kit expressed by stem cells, etc.) with a second mAb directed at antigens present at the site of injury (*i.e.* fibrinopeptide A, VCAM, etc.). The resulting bispecific antibodies (BiAb) will then be used to target a particular cell population to an injured tissue. We will study the trafficking of targeted cell populations to injured tissue in two murine models of wound repair; a full thickness tail-wound, and an external muscle crush injury. Finally, we will measure the effects of BiAb-mediated targeting of specific cell populations to these injured tissues on tissue repair and function. Upon proof of principle for these uses of BiAb technology, a wide variety of clinical applications in regenerative medicine are envisioned limited only by the identification of targeted cell- and injured tissue-specific antigens.

Wolfgang Peti, PhD
Assistant Professor of Medical Science, Bio Med Molecular Pharmacology, Physiology & Biotechnology, Brown University
EPSCoR

"Structural Basis for Protein Phosphatase 1 Regulation"

Despite exceptional and enduring research efforts to understand the regulation of Protein Phosphatase 1 (PP1), only incremental progress has been reported. It is well-known that the common PP1 binding motif (R/K-$X_1X_2X_3$-F/W) pocket, a motif required in the more than 80 identified PP1 regulatory proteins, is more than 20 Å away from the PP1 active site. This requires that *PP1 inhibitory proteins* either (a) be extremely large (when folded) or (b) have high intrinsic flexibilities in order to bind both the motif binding pocket and active site simultaneously. Using NMR spectroscopy, we have characterized the PP1 pseudo-substrate inhibitor darpp-32 (darpp-32 needs to be phosphorylated at Thr34 to become a potent inhibitor), a central PP1 inhibitor in neurons, and the ubiquitous inhibitor-2, indicated to play a critical roles in cell cycle regulation. Our NMR investigations show that both inhibitors fall into the second category, sometimes also referred to as intrinsically disordered proteins. Interestingly, our NMR-based analysis, which includes local chemical shift analysis, fast time-scale dynamics measurements and long-range paramagnetic tag studies, shows that despite their high intrinsic flexibilities, the unbound inhibitors show preferred local and 3-dimensional conformations. Our data describes how these preferred conformations likely encode for the specific interaction patterns of these PP1 inhibitors for PP1 and are in outstanding agreement with the recently reported complex structure between PP1:Inhibitor-2.

Notably, these characteristics of these inhibitor proteins are very different from those we observed for spinophilin, which, to date, is the first *PP1 targeting protein* characterized in its unbound state. Spinophilin targets PP1 to its points of action within the cell, including the F-actin cytoskeleton and glutamatergic receptors, among others. Based on our NMR analysis, we show that the PP1 targeting domain of spinophilin behaves like a random-coil polymer in solution, without any local or long range preference, in striking contrast to what we observed for the PP1 inhibitory proteins. In addition, this behavior of the spinophilin PP1 targeting domain (spinophilin residues 417-494) is not changed when it is fused to the immediately C-terminal spinophilin PDZ domain (fusion construct includes spinophilin residues 417-602).

In order to understand the molecular details of the spinophilin:PP1 interaction, we determined the 1.8 Å crystal structure of the PP1:spinophilin complex, the most important PP1 holoenzyme in neurons. Spinophilin's high intrinsic flexibility in solution allows it to wrap in an intimate and extended fashion upon binding PP1. This results in an extensive interaction surface between the two proteins, resulting in strikingly different biochemical characteristics than observed for PP1 alone. Importantly, the structure, which is radically different from that of the PP1:MYPT1 complex, the only other known PP1:targeting protein complex, also demonstrates that the binding of PP1 targeting proteins to PP1 are highly individual and cannot be easily classified. Finally, the PP1:spinophilin complex structure allows us to readily interpret and understand, at a molecular level, the considerable amount of biochemical data that has been generated for this complex.

In summary, this structural data obtained for PP1 inhibitor and PP1 binding proteins in both their unbound (darpp-32, inhibitor-2 and spinophilin) and bound (spinophilin:PP1) states enables us to provide much novel insight into the structural basis of PP1 regulation by its multiple interacting proteins.

POSTER COMPETITION

5th Level – Rotunda Lobby from 12:45 pm to 3:00 pm.

There will be three classes of competition for posters: Junior Investigator, Trainee, and Collaborative Research Project.

POSTER JUDGES

Clyde Briant, PhD
Poster Session Chair
Vice President, Research
Brown University
Co-chair
RI Science & Technology Council

Deborah Britt, PhD
Assistant Professor of Medicine (Research)
Brown University
Deputy Director
COBRE for Cancer Research Development
Rhode Island Hospital

Gregory Jay, MD
Assistant Professor of Emergency Medicine
Brown University
COBRE for Skeletal Health and Repair
Rhode Island Hospital

Surenda Sharma, PhD, MBBS
Professor of Pediatrics
Brown University
Deputy Director
COBRE for Perinatal Biology
Women & Infants Hospital

Marshall Kadin, MD
Professor of Dermatology
Boston University School of Medicine
COBRE for New Approaches to
Tissue Repair
Roger Williams Medical Center

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Bloom Syndrome Protein: Production and Purification of Fragments to Study Protein Partnerships

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Bloom syndrome is a rare autosomal recessive disorder characterized by photosensitivity, an increase to respiratory illnesses, and premature death due to a predisposition to a wide array of cancerous states. The BLM gene is part of the RecQ helicase family. An increase in sister chromatid exchange serves as markers for genomic instability and is used to diagnose Bloom Syndrome however; there is currently no known cure. Bloom Syndrome is caused by mutations of the BLM gene. The Almeida lab focuses on the protein partnerships of BLM with proteins known to influence genomic stability. Research suggests that the N- and C-termini of BLM may affect stability by partnering with the recombination repair protein Rad51. Therefore, a systematic set of deletion mutants have been generated for the termini. DNA from each polypeptide fragment was cloned into Gateway entry vectors, sequenced and recombined into destination vectors for expression in *E. coli*. The resulting oligopeptide fragments contain both a 6x Histidine epitope tag on the N-terminus and an engineered Flag epitope tag on the C-terminus that are used for purification and protein-protein interaction analysis via co-immunoprecipitation . Nickel affinity column using the BioLogic LP system was used to enrich each fragment. Co-IP analysis using Nickel as solid support will then determine the strength of each fragments partnership with Rad51. Analysis of this protein-protein interaction will lead to a better understanding of the role of BLM in genomic stability.

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A2

CORRELATION OF LAT-1 GENE EXPRESSION AND PLACENTAL IMPLANTATION DISORDERS

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The LAT-1 gene, originally discovered in cancerous tissue, has been shown to be highly expressed in the human placenta. The placenta and some cancers are very similar in their invasive and proliferative properties. Cancerous cells invade into a variety of healthy tissues. Likewise, trophoblast cells (which make up the embryonic placenta) invade into the wall of the uterus during implantation of the placenta. The LAT-1 gene encodes an amino-acid transporter, partly responsible for signaling to the embryo when conditions are right for implantation. Defects in the expression of this gene may well be correlated with various implantation disorders that affect women. Failure of normal placental implantation inhibits the exchange of nutrients and waste products between a mother and her fetus. This can cause growth restriction in the fetus and complicate the pregnancy, often resulting in preterm birth. It is possible (likely) that the failure of trophoblast cells to adequately invade and remodel the maternal decidua is correlated with a sub-normal level of LAT-1 expression. The up-regulation of this L-amino acid transporter also coincides with invasion and implantation in the mouse. We examined LAT-1 cell specific expression from early and term placentas of healthy and preeclamptic pregnancies. LAT-1 expression is most abundant in invasive extravillous trophoblasts and syncytiotrophoblasts on placenta villi. Quantitative morphometry is in progress. This pattern of expression may be part of the phenotypic change in trophoblast cells with increased cell motility and invasion characteristic of early trophoblast cells. Its dysregulation may underlie defects in implantation (preeclampsia or placenta accreta).

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A3

Total Liver Isolates Transplanted into Mitomycin C Treated Rats Yield Donor Colonies of Donor Endothelial Cells, Hepatocytes and Bile Ducts

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Background: Pretreatment of rats with retrorsine crosslinks host hepatocyte DNA and prevents proliferation after partial hepatectomy (PH), allowing selective expansion of transplanted progenitors. Shortcomings of this method are the length of the protocol (7 weeks) and the carcinogenicity and limited availability of retrorsine.
Methods: This report describes a rapid protocol using mitomycin C (MMC) for promoting expansion of transplanted progenitor cells. To promote donor cell expansion, host dipeptidyl peptidase IV negative (DPPIV-) Fischer rats were injected intrasplenically with MMC. After 7/8 days, donor liver isolates from DPPIV+ Fischer or ACI rats were infused into the host liver via the spleen. For allogeneic liver isolates, host rats were injected with anti CD3 antibodies daily for 4 days. Two weeks of antibiotic treatment was initiated 24-hours post-surgery
Results: Histochemical staining of host rat livers harvested 2-9 weeks after syngeneic or allogeneic transplantation revealed well-defined donor hepatocyte colonies with strong canalicular DPPIV activity. These hepatocyte colonies also co-expressed membrane and cytoplasmic marker proteins characteristic of adult hepatocytes. In addition, host livers contained groups of sinusoids lined by endothelial cells co-expressing DPPIV and the endothelial cell marker, RECA-1. Mixed colonies containing donor hepatocytes, endothelial cells and infrequently, bile ducts, were also observed.
Conclusions: The MMC transplantation model will provide a rapid method for investigating interactions *in vivo* between hepatocytes and endothelial cells, for assessing the role of endothelial cell chimerism in allogeneic transplantation and for evaluating the ability of both syngeneic and allogeneic hepatic progenitors to engraft, expand and differentiate in adult rat livers.

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A4

Effect of Akt1-Deficiency on Female Fertility in Mice

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Problem: Early pregnancy success is regulated by the exquisite integration and coordination of numerous physiological processes at the molecular level. We hypothesize that Akt1 functions to maintain a homeostatic balance between cell survival and apoptosis in the female reproductive tract.
Methods: Akt1 wild type and Akt1-deficient female mice were mated to wild type males of proven fertility to determine both the average number of litters per female and the number of pups per litter. In order to determine if Akt1-mediates FSH growth and/or maturation of the ovary, experiments to determine estrous cycle, hormone levels and the number of follicles following PMSG-stimulation of prepubertal Akt1 wild type and Akt1-deficient female mice were conducted.
Results: Akt1-deficient females were significantly less fertile relative to their wild type counterparts. Akt1-deficient females exhibited an extension of the diestrous phase. Data showed that Akt1-deficiency has a deleterious effect on FSH levels, as these mice had significantly lower serum concentrations. Fewer mature follicles were observed in Akt1-deficient females following PMSG-stimulation.
Conclusions: Our data shows a decrease in the number of mature follicles in the Akt1-deficient mice at the point in maturation in which FSH is required for progression. We propose that Akt1 has a particular effect on FSH levels and the subsequent FSH-mediated maturation of ovarian follicles. The dysfunction in cyclicity strongly supports the concept that Akt1 plays a critical role in the homeostatic maintenance of the female reproductive tract.

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A5

Genetic Disruption of Protein Kinase C (PKCδ) Reduces Sepsis-Induced Lung Injury. B Casserly[1,2], KL Grinnell[1,2], J Lomas-Neira[3], C Chung[3], A Ayala[3], S Rounds[1,2], JR Klinger[1,2], EO Harrington[1,2]. *Vascular Research Laboratory[1], Providence Veterans Affairs Medical Center, Departments of Medicine[2] and Surgery[3], Warren Alpert Medical School of Brown University, Providence, RI 02908*

Pulmonary sequestration of circulating neutrophils is well characterized in lipopolysaccharide (LPS)-induced acute lung injury (ALI) and central to the pathogenesis of the adult respiratory distress syndrome (ARDS). However, the intracellular signaling pathways responsible for neutrophil migration to the lung following LPS stimulation are unknown. PKCδ has been demonstrated to play a central role in neutrophil apoptosis and thrombin-induced ICAM-1 gene expression in endothelial cells. To test the hypothesis that PKCδ modulates pulmonary inflammatory responses in ALI, we examined pulmonary permeability and lung neutrophil migration responses to LPS in wild-type and PKCδ knockout mice. Under baseline conditions, no differences were seen in filtration coefficients (k_f) between wild-type and PKCδ knockout mice. Similarly, there was no difference in wet to dry weights in wild type and knockout mouse lungs at baseline or in response to hydrostatic challenge. However, 24h after intraperitoneal injection of LPS, k_f was significantly lower in lungs isolated from PKCδ knockout mice relative to wild type, suggesting an attenuation of LPS-induced injury. Neutrophil transmigration was also reduced in lungs from PKCδ knockout mice compared to wild type lungs exposed to LPS. This data supports a central role for PKCδ in the cellular and chemokine inflammatory responses following exposure to LPS stimulation and a potential therapeutic target in attenuating lung injury that commonly complicates infection and sepsis. Possible mechanisms, including delayed neutrophil apoptosis, altered cytokine production and inflammatory cell proliferation, remain to be elucidated.
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A6

The Utility of the DNA Microarray Scanner to Simplify the Immunofluorescence Evaluation of Autoimmune Bullous Diseases

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Problem: The current method of diagnosing autoimmune bullous diseases relies on direct immunofluorescence microscopy. This method is fraught with limitations imposed upon the user, such as the requirement of a dark room, difficulties associated with the small field of view of a microscope, lower signal production from the standard fluorophore fluorescein, and the need for an experienced technician.
Method: A DNA microarray scanner was used as a digital fluorescence microscope for diagnosing autoimmune bullous diseases. Frozen sections of skin biopsies were taken from three patients with bullous pemphigoid, and one patient each with lichen planus pemphigoides, linear IgA disease, and dermatitis herpetiformis. After incubation with cyanine labeled antibodies, the tissues were scanned at five-microns resolution using a tool originally designed to study gene expression.
Result: The microarray scanner's large field of view, unlike that of fluorescence microscopy, allowed a view of the entire specimen. All images were diagnostic and included a linear pattern along the basement membrane zone using anti-IgG and anti-C3 in all cases of bullous pemphigoid; a linear pattern of IgG along the BMZ in lichen planus pemphigoides; and a linear pattern of IgA along the BMZ in linear IgA dermatosis. IgA deposition along dermal papillary tips was seen in dermatitis herpetiformis.
Conclusion: The advantages of the microarray scanner over standard fluorescence microscopy include the technical ease, the large field of view simplifying the orientation of tissue, the potential for visualizing multiple antibodies simultaneously in a tissue, and the convenience of digital image archiving.

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A7

LAT-1 EXPRESSION IN PLACENTAL INVASION AND DEVELOPMENT

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Objectives: LAT-1 (L-type amino acid transporter 1) is a system L, Na+-independent amino acid transporter responsible for transport of large neutral amino acids. Dysregulated expression of LAT-1 is characteristic of many primary human cancers and is related to tumor invasion. Primary rat hepatocytes in culture increase LAT-1 mRNA in response to amino acid depletion. Transformed hepatic cell lines demonstrate constitutive expression of LAT-1. These observations suggest that LAT-1 expression confers a growth and survival advantage under limited amino acid availability. LAT-1 is highly expressed in the placenta. It has been shown previously that amino acids are fundamental regulators of cell function and energy metabolism in pre-implantation embryos. Our objectives were to analyze qualitatively and quantitatively LAT-1 expression in pre-implantation stages of mouse embryo development and to identify cell types expressing LAT-1 in post implantation stages. Methods: LAT-1 was quantified by Northern blot and real-time qPCR. Localization of expression was by laser capture microdissection and *in situ* hybridization. Results: Our results show increasing mRNA levels of LAT-1 as the embryo develops from zygote to blastocyst and increasing mRNA levels as placenta develops from gestation day 7.5 to13.5.Expression studies of LAT-1 on microdissected samples from developing mouse placenta show highest levels of LAT-1 mRNA in trophoblast giant cells at the time of implantation (E7.5). Conclusions: Since trophoblast giant cells are invasive cells that displace and phagocytose the uterine epithelial cells, these data suggest that LAT-1 may play a role in the invasive phenotype. The mechanism of LAT-1 regulation during placentation, therefore, might provide valuable clues to its role in tumor progression and invasion.

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A8

INSTRUMENTATION AT THE RHODE ISLAND NSF/EPSCoR PROTEOMICS FACILITY

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The Rhode Island NSF/EPSCoR Proteomics Facility, located in the Laboratories for Molecular Medicine at Brown University, houses an assortment of state-of-the-art equipment for the biochemical and biophysical characterization of proteins and other macromolecules. The facility maintains, and offers to the entire Rhode Island research community, the following instruments:

- Jasco J-815 Circular Dichroism Spectrometer with Peltier heating
- MicroCal VP-DSC Differential Scanning Microcalorimeter
- MicroCal VP-ITC Isothermal Titration Microcalorimeter
- Biacore T-100 Surface Plasmon Resonance
- Thermo-Finnigan LTQ Mass Spectrometer with LC-ESI
- Horiba Jobin Yvon FluoroMax-4 Spectrofluorometer
- Qiagen QIAcube
- Infors Minifors Fermentor
- Agilent 1200 series HPLC with Autosampler, UV Detector and Fraction Collector
- GE Healthcare Akta Prime Plus Liquid Chromatography System

Examples of data collected by researchers in the facility will be provided, demonstrating the utility of various instruments. In addition to extending access to the instruments, another mission of the facility is to deliver training to users and consultation on experiments. Training is in the form of online tutorials and hands-on instruction at the facility. Consultations on experimental design and the capabilites of various instruments are offered to all Rhode Island scientists via phone, email and direct meetings.
More information about the facility, including instrumentation, protocols, funding opportunities and a sign-up calendar, are available at http://biomed.brown.edu/epscor_proteomics.

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A9

THE LEDUC BIOIMAGING FACILITY

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The Leduc Bioimaging Facility, open to all investigators, provides equipment and training dedicated to high-resolution imaging in the life sciences. The facility operates on a fee-for-service basis and offers its services in the Laboratories for Molecular Medicine and Sidney Frank Hall for Life Sciences. The facility in the Laboratories for Molecular Medicine includes a Zeiss LSM410 confocal laser scanning microscope, a Zeiss Axiovert 200M fluorescence microscope, and MetaMorph 7.0 image analysis software. A new confocal microscope will be set up in the Laboratories for Molecular Medicine in 2008. The facility in Sidney Frank Hall includes a Philips 410 transmission electron microscope, a Hitachi 2700 scanning electron microscope, a Zeiss Axiovert 200M fluorescence microscope, a Zeiss Lumar fluorescence stereomicroscope, a Leica TCS SP2 AOBS confocal laser scanning microscope, a Zeiss LSM510 Meta confocal laser scanning microscope, and MetaMorph 7.0 image analysis software. The facility in Sidney Frank Hall also maintains equipment for sample preparation, including a critical point dryer, sputter coater, and microtomes for ultrathin sectioning.

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A10

TOYS AND TECHNOLOGIES FOR PEDIATRIC UPPER EXTREMITY REHABILITATION

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Problem: Brain injury, cerebral palsy and other neurological conditions create profound movement disorders in children, often leading to impaired use of their arm. Rehabilitation with physical therapy is critical for functional recovery and play is the ideal means to provide this therapy. Our goal was to design and build a line of toys that are actually rehabilitative devices.
Methods: We established collaborations across the state between businesses, academic and clinical initiations. Four toys were designed with a constant theme - they are supplemental physical therapy, as well activity monitors. The toys are actually controllers of commercially available toys, designed to require specific wrist and forearm motions for play. Design requirements included programmable range of motion, gain, data logging, and downloaded capabilities.
Results: Four toy models have been designed and built. Power Glove: Controls a wide range of remote controlled cars and toys through a glove that maps wrist flexion/extension and forearm supination/pronation motion to the toy's controlled functions. The PowerGlove provides children with the greatest mobility and will enable children to receive independent rehabilitative therapy for cerebral palsy or hemiplegia at any given location. FE SCC: Controls HO slot car speed through wrist flexion/extension motion. SP SCC: Controls HO slot car speed through forearm supination/pronation motion. Computer Switch: Controls switching in computer games through wrist and forearm motion. Children turn/rotate the switch to activate.
Conclusions: Toys were designed and built to provide and record supplemental physical therapy. Patients are currently being enrolled in a clinical study to assess the rehabilitative efficacy of these toys.

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A11

FATE AND EFFECTS OF ADULT BONE MARROW CELLS IN LUNGS OF NORMOXIC AND HYPEROXIC NEWBORN MICE

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Problem: Cell-based therapy in adult lung injury models is associated with highly variable donor cell engraftment and epithelial reconstitution. The role of marrow-derived cell therapy in neonatal lung injury is largely unknown. In this study, we determined the fate and effects of adult bone marrow cells in newborn lungs.
Methods: Normoxic or hyperoxic (95% O_2) wild-type mice received unfractionated bone marrow cells from animals expressing green fluorescent protein (GFP) at postnatal day 5 (P5). Controls received vehicle buffer. Lungs were analyzed between post-transplantation (TPX) day 2 and week 8.
Results: The volume of GFP-immunoreactive donor cells, monitored by stereological volumetry, remained constant between post-TPX weeks 1 and 8 and was similar in normoxic and hyperoxia-exposed recipients. Virtually all marrow-derived cells showed colocalization of GFP and the pan-macrophage marker, F4/80, by double immunofluorescence studies. Epithelial transdifferentiation was not seen. Marrow cell administration had adverse effects on somatic growth and alveolarization in normoxic mice, while no effects were discerned in hyperoxia-exposed recipients. Reexposure of marrow-treated animals to hyperoxia at P66 resulted in significant expansion of the donor-derived macrophage population.
Conclusions: Intranasal administration of unfractionated bone marrow cells to newborn mice does not achieve epithelial reconstitution but establishes persistent alveolar macrophage chimerism. The predominantly adverse effects of marrow treatment in newborn lungs are likely due to macrophage-associated paracrine effects. While this model and route of cell therapy may not achieve epithelial reconstitution, the role of selected stem cell populations and/or alternate routes of administration for cell-based therapy in injured newborn lungs deserves further investigation.

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A12

MODULATION OF THE P53 RESPONSE BY UNCOUPLING PROTEIN-2 PROMOTES CHEMORESISTANCE IN COLON CANCER CELLS

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Problem: Cancer cells acquire chemoresistance by selection pressure in response to an unfavorable microenvironment. This process is modulated by oxidative stress that primarily originates from mitochondria. We studied if this adaptive response in cancer cells is linked to uncoupling protein-2 (UCP2), a regulator of mitochondrial reactive oxygen species (ROS) production with enhanced expression in human colon cancer.
Methods: We overexpressed UCP2 in HCT116 colon cancer cells and analyzed the effect of various chemotherapeutics in vitro and in vivo. We monitored the impact of UCP2 on ROS levels and apoptosis after camptothecin (CPT), etoposide, cisplatin, and doxorubicin treatment. Additionally, we generated subcutaneous xenografts by inoculating UCP2 overexpressing HCT116 cells into nude mice and monitored tumor growth in response to irinotecan.
Results: UCP2 overexpression lowered ROS levels and reduced apoptosis in HCT116 cells treated with chemotherapeutics. This protection by UCP2 was absent in p53-/- HCT116 cells. UCP2 overexpression diminished CPT-induced N-terminal phosphorylation of p53 and increased its proteasomal degradation. Additionally, inhibition of glycolysis, a novel metabolic function of p53 was altered: UCP2 overexpressing HCT116 cells exhibited higher lactate production and increased susceptibility to glycolysis inhibitor 2-deoxyglucose. The growth inhibitory effect of irinotecan was diminished in mice receiving xenografts of UCP2 overexpressing cells.
Conclusions: UCP2 has a protective effect in colon cancer cells treated with chemotherapeutics and this response involves modulation of p53 response and promotion of glycolytic phenotype. The association between increased UCP2 expression and chemoresistance suggests that UCP2 may become a novel target in the treatment of colon cancer.

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A13

AN AKT1-MEDIATED GERM CELL SURVIVAL RESPONSE INVOLVES γ-H2AX SIGNALING

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Problem: Targeting of the PI3K/Akt signaling pathway has been linked to increased radio- and chemo-sensitivity. A better understanding of the role of this pathway in the testis is essential for improved therapeutic approaches to cancer, as well as to provide further insight into normal testicular homeostasis. This study assesses Akt dependant germ cell apoptosis following x-irradiation *in vivo* and *in vitro*.
Methods: The expression and localization of two independent DNA double strand break (DSB) repair factors, phosphorylated (γ) H2AX and p53-binding protein 1 (53BP1) were examined *in vivo* as well as in the spermatogonial germ cell line GC-1, as the Akt1-deficient mitotic spermatogonia were found to be the most sensitive to x-irradiation induced injury.
Results: γ-H2AX protein remained elevated in Akt1-deficient testes following x-irradiation relative to wild type testes. In control testes, γ-H2AX staining was observed in spermatogonia and the XY body of spermatocytes. Following x-irradiation, γ-H2AX formed discrete foci in round spermatids and spermatocytes and as an intense staining pattern in spermatogonia. Addition of the selective PI3K inhibitor, LY29004, and X-ray triggered a robust increase of apoptosis in GC-1 cells when compared to radiation or inhibitor alone.
Conclusions: taken together, our results indicate that 53BP1 and γ-H2AX are differentially expressed in various types of germ cells both before and after damage, and suggest accumulation of γ-H2AX contributes to the radio-sensitivity exhibited by Akt1-deficient germ cells, thereby linking cell survival to components of the DNA damage surveillance machinery.

This work was supported by a Biomedical Research Infrastructure Network/IDeA Network of Biomedical Research Excellence Grant No. P20 RR016457 from the National Center for Research Resources/National Institutes of Health.

A14

DEVELOPMENTAL ARREST IN BRD2-DEFICIENT MICE

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Problem: Hierarchical genetic cascades drive the process of embryonic development. The understanding of these complex, layered genetic interactions essential to producing a viable organism are further complicated by the contextual environment of genes in chromatin. Mechanistic description correlating the control of gene expression to the genesis of anatomical structures is the newest challenge of developmental biology.
Methods: A gene-trapped embryonic stem cell line was utilized to establish a mouse model deficient in Brd2. Embryos were characterized morphologically using standard histological techniques and molecularly using quantitative PCR.
Results: Brd2 deficiency results in embryonic lethality at E10.5 manifested by gross growth and morphological abnormalities and neural tube closure defects. Gene expression profiling revealed disregulation of both general cell cycle regulators and factors associated with specification and regionalization of the central nervous system.
Conclusions: These results establish Brd2 as an essential protein for mammalian neurulation and provide a model system for the further study of the mechanistic connection between Brd2 and neurodevelopment.

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A15

FUNCTIONAL INTERACTION BETWEEN FANCD2 AND PCNA *VIA* A CONSERVED PCNA-INTERACTION MOTIF

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Problem: Fanconi Anemia (FA) is a rare recessive disorder characterized by hematological abnormalities, developmental defects and elevated cancer risk. FA cells are highly sensitive to DNA cross-linking agents, e.g. mitomycin C (MMC). The FA pathway is activated by DNA damaging agents and during S phase of the cell cycle *via* the mono-ubiquitination of the FANCD2 and FANCI proteins, signaling their translocation to nuclear foci where they co-localize with DNA replication and repair proteins. We have characterized the interaction between FANCD2 and PCNA, the major cellular DNA polymerase processivity factor and have identified a highly conserved PCNA-interaction motif (PIP-box) in FANCD2.
Methods: Using site-directed mutagenesis we have mutated the critical FANCD2 PIP-box amino acids, and introduced mutant FANCD2 PIP∆ into an FA-D2 patient-derived cell line. Results: Unlike wild-type FANCD2, FANCD2 PIP∆ fails to efficiently interact with PCNA but retains the ability to interact with the FA core complex component FANCE. FANCD2 PIP∆ fails to become mono-ubiquitinated spontaneously as well as following exposure to DNA damaging agents, and fails to accumulate in discrete nuclear foci. However, like wild-type FANCD2, FANCD2 PIP∆ is phosphorylated following exposure to X-irradiation. Consequently, FANCD2 PIP∆ fails to correct the MMC sensitivity, MMC-induced G2 accumulation, and the elevated DNA damage-induced chromosome breakage of FA-D2 patient-derived fibroblasts.
Conclusions: Our results strongly suggest that FANCD2 associates with PCNA in a DNA damage surveillance capacity. Thus, PCNA promotes the localization of FANCD2 to sites of DNA damage, and functions as a molecular platform to facilitate the mono-ubiquitination of the FANCD2 protein during the DNA damage response.

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A16

Use of monolithic chromatographic supports for removal of highly abundant plasma proteins

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During process development for the detection of biomarker candidates, highly abundant plasma proteins, such as human serum albumin (HSA) and IgG, are frequently removed from human plasma or serum. Removal of these proteins is frequently time consuming, and expensive resins have to be used. Loss of HSA-interacting proteins and possible contamination, caused by “carry-over” from previous chromatographic runs, are frequent biases when this technology is used.
We have developed a simple and rapid method for the separation of IgG and HSA from proteins with lower abundance by use of a miniature monolithic weak anion-exchange column. In a second step, the low abundance proteins are further fractionated on a strong monolithic anion-exchange column. Separated proteins have been analyzed by SDS-PAGE and identified by LC-MS/MS.
Our results indicate that use of this pre-fractionation method combined with LC-MS/MS is a powerful tool for the identification of low abundance therapeutic proteins, such as clotting factors and inhibitors. Some biomarker candidates with very low abundance were also detected.

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A17

DECREASED LUBRICIN CONCENTRATIONS AND MARKERS OF JOINT INFLAMMATION IN SYNOVIAL FLUID OF PATIENTS WITH ANTERIOR CRUCIATE LIGAMENT INJURY

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Problem: To study the effort of anterior cruciate ligament (ACL) injury on lubricin concentration in synovial fluid (SF) and its correlation with time postinjury, inflammatory cytokines, lubrcin-degrading enzymes, and SF proteoglycan content.

Methods: SF samples were obtained from both knees of 30 patients with a unilateral ACL insufficiency 32-364 days postinjury. Lubricin, inflammatory cytokines (interleukin-1β [1L-1 β], tumor necrosis factor α [TNFα], and IL-6) and catabolic enzymes (procathepsin B and neutrophil elastase) were quantified in the SF of injured and contralateral (uninjured) joints using enzyme-linked immunosorbent assays. Sulfated glycosaminoglycan (sGAG) levels in the SF were measured by Alican blue binding assay.

Results: SF lubricin concentrations were significantly ($P < 0.001$) reduced following ACL injury when compared with those in the contralateral joint. Within 12 months, the lubricin concentration in the injured knee (slope = 0.006, SE = 0.00010, $P < 0.001$) approached that in the contralateral knee, which did not change with time (slope = -0.0002, SE = 0.00050, $P = 0.71$). TNFα levels showed a significant negative relationship with $\log_2$ lubricin levels. IL-1β, TNFα, IL-6, procathepsin B, and neutrophil elastase concentrations in SF from injured knees were greater in samples from recently injured knees compared with those that were chronically injured. There were no detectable cytokines or enzymes in the SF of contralateral joints. Concentrations of sGAG were significantly ($P < 0.01$) higher in the SF from injured knees compared with the contralateral joints.

Conclusions: The decrease in SF lubricin concentrations following ACL injury may place the joint at an increased risk of wear-induced damage as a consequence of lack of boundary lubrication, potentially leading to secondary osteoarthritis. The decrease in SF lubricin was associated with an increase in inflammation.

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A18

Elevated heme and IL-10 deficiency trigger pre-eclampsia like symptoms

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Objective: Although several anti-angiogenic biomarkers have been recently identified, little is known about the upstream regulatory factors that initiate the onset of pre-eclampsia (PE)-associated symptoms. We hypothesize that free heme triggers intrauterine complement activation, resulting in PE like symptoms and release of anti-angiogenic factors.
Methods: Serum levels of heme from normal and PE patients were analyzed by colorimetric measurements. Heme deposition in placental sections was evaluated by IHC. To evaluate the *in vivo* effects, pregnant wild type or IL-$10^{-/-}$ mice were injected i.p. with hemin (0, 5, 12.5 or 25mg/kg) on gd 10. Blood pressure (BP), proteinurea, serum sFlt-1 and sEng, and heme were monitored on gd 17. Placental C3 deposition and kidney pathology were analyzed. Dual cell tube formation as well as molecular analysis of VEGF isoforms were studied *in vitro*.
Results: Increased placental deposition of heme was observed in severe PE cases. In an experimental model, heme treatment significantly elevated BP, proteinuria, sEng, sFlt-1 and triggered C3 deposition in IL$10^{-/-}$ mice. In wild type mice, despite proteinuria and IUGR, no change was observed in BP. *In vitro* heme treatment inhibited angiogenesis due to reduced expression of VEGF C that was reversible with IL10.
Conclusions: Our results imply that free heme is an inflammatory trigger for PE. These observations are important in that PE has been shown to be associated with reduced expression of heme oxygenase-1. Genetic predisposition, as seen in IL-$10^{-/-}$ mice, may augment the manifestation of PE when coupled with inflammatory triggers such as heme.

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A19

ALLOSTERIC MODULATION OF SULT2A1 BY CELECOXIB AND NIMESULIDE: COMPUTATIONAL ANALYSES

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Problems: SULT2A1 catalyzes sulfonation of estradiol (E_2) at the 3-OH-position under normal conditions. In the presence of celecoxib, sulfonation of E_2 is altered allosterically to favor the 17β-OH-position. This is noteworthy because 3-OH sulfonation allows regeneration of E_2, while 17β-OH sulfonation is expected to decrease E_2 levels. Our aims were to identify the location of the allosteric binding site for human SULT2A1 and elucidate the structural interactions which cause 'switching' of sulfonation from 3-OH-E_2 to 17β-OH-E_2 by celecoxib. We also investigated the potential for allosteric modulation of SULT2A1 by nimesulide.
Methods: Binding cavity was calculated using Accelrys DiscoveryStudio 2.0. Docking analyses were conducted with AutoDock4. Protein-ligand complexes were energy-minimized with the CHARMM module of DiscoveryStudio.
Results: Binding site analyses demonstrated that the substrate and cofactor binding cavities of SULT2A1 are connected by a narrow channel. Our docking studies found that the substrate cavity can bind E_2 and modulator simultaneously. When we docked E_2 in absence of celecoxib, 100% of the total E_2 conformations oriented with the 3-OH in position for sulfonation. In the presence of celecoxib, E_2 docked with either 17-OH in position for catalysis or in non-catalytic positions. In the presence of nimesulide, E_2 favorably docked in non-catalytic positions.
Conclusions: The docking experiments showed that both celecoxib and nimesulide can alter the E_2 position noticeably, favoring catalysis at the 17β-OH-position or no catalysis at all. Celecoxib and nimesulide preferred to dock to the space where E_2 was bound forcing E_2 to bind to an alternative region in the cavity.

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A20

STRUCTURAL DIVERSITY AND SIMILARITY AMONGST SULFOTRANSFERASES: HUMANS TO TICKS AND BEYOND

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Problems: Due to the explosion of genomic and proteomic techniques, hundreds sulfotransferase enzymes have been identified from diverse taxa including vertebrate, invertebrate, plant, bacteria, and fungal species. Even though these enzymes retain relatively low overall sequence similarity, certain signature regions across the sulfotransferase domain are highly conserved, and the protein-fold is highly conserved from bacteria to plant to human forms. Our aim was to use detailed structure and sequence comparisons across diverse taxa to identify

Methods: We used Accelrys SeqLab for sequence analyses; and we used Accelrys Discovery Studio and UCSF Chimera for structural analyses. Structures were obtained from RCSB ProteinDataBank, and sequences were obtained from Protein Family (Pfam) database.

Results: We combined analysis of 36 crystallographic sulfotransferase structures, amino acid sequence alignments, and known function to describe conserved primary sequence motifs for cofactor binding, substrate binding, and catalytic residues for cytosolic and membrane-bound sulfotransferases from over 100 diverse species. In addition, we apply these characteristic structural motifs to make functional predictions for two new sulfotransferases expressed in the salivary gland of *Ixodes scapularis* (black tick).

Conclusions: This is the first use of modern bioinformatics tools to compare sequence and structure amongst diverse sulfotransferases with application to characterization of novel sulfotransferases.

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A21

NOVEL SULFOTRANSFERASE FROM BLACK-LEGGED TICK SULFONATES DOPAMINE AND OCTOPAMINE

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Problem: The Black-legged tick (*Ixodes scapularis*) is the major vector of Lyme disease in the Northeastern United States. cDNA transcripts of two novel sulfotransferases have been identified in the salivary gland, and we hypothesized that these sulfotransferases may sulfonate one or more host or tick molecules important for modulating the host immune system, the host coagulation response or the tick salivation process, resulting in changes in feeding success or tick-host pathogen transmission.
Methods and Results: We used bioinformatics approach to determine that both tick sulfotransferases were sequence-related to the cytosolic superfamily of sulfotransferases (SULT) which catalyzes sulfonation of small molecule phenols and alcohols including steroids and monoamine neurotransmitters. Expression analysis determined that mRNA and protein levels of *Ixosc* Sult 1 and Sult 2 were modified by feeding. Using tissue homogenate and recombinant expressed Sult 1 or Sult 2, we found that dopamine and octopamine could serve as substrates.
Conclusions: *Ixosc* Sult 1 and Sult 2 may serve as critical modulators of dopamine-dependent prostaglandin synthesis and salivary secretion, serving to inactivate the signal for salivary secretion in *Ixodes scapularis*.

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A22

RHODE ISLANDERS' ATTITUDES TOWARD THE DEVELOPMENT OF A STATE-WIDE GENETIC BIOBANK

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Aims: To explore the attitudes of Rhode Island residents toward the potential development of a large, prospective, population-based genetic Biobank to store blood for future biochemical and molecular analyses, and to gauge their willingness to participate in a prospective population-based study of sudden cardiac arrest.
Methods: A mailed survey and focus groups.
Results: Survey respondents and focus group participants indicated willingness to provide biospecimens, medical history and personal lifestyle information, and to undergo medical tests. Datasets from both respondent groups included multiple concerns about long-term storage of biospecimens and personal information, and the need of potential Biobank participants for detailed information about study protocols and oversight.
Conclusions: A population-based Biobank has great potential for successful participant recruitment in Rhode Island; however, adequate attention to the public's concerns and questions is an essential preparatory step.

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A23

ROLE OF RGS9-2 IN SCHIZOPHRENIA AND PARKINSON'S DISEASE PHARMACOTHERAPY.

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Problem: Drug-induced dyskinesia (DID) are unexplained and irreversible movement side-effects of the pharmacotherapy of Parkinson's disease and schizophrenia, and are thought to result from the chronic actions of the respective drugs on striatal D2-like dopamine receptors (D2R). RGS9-2 is a member of the RGS family of Gα GTPase accelerating proteins that is specifically expressed in the striatum and the objective of the study was to identify a role for RGS9-2 in DID.
Methods: Using appropriate drug-treatment paradigms we evaluated if RGS9 knockout mice developed abnormal movements that resembled DID. We examined the cellular distribution pattern of RGS9-2 and D2R in striatal neurons and asked if RGS9-2 could functionally modulate D2R evoked signals in these cells. We also evaluated if RGS9-2 and D2R interacted when the two proteins where expressed together *in vitro*. Finally, we evaluated if RGS9 polymorphisms in human patients could predict risk for developing DID.
Results: We found that RGS9-2 and D2R have identical cellular distribution patterns in striatal neurons and D2R abnormally inhibits glutamate-elicited currents in striatal neurons from RGS9 knockout mice. In addition, RGS9 knockout mouse develop drug-induced abnormal movements that more closely resemble DID than in any other rodent model. RGS9-2 can target to G protein coupled D2R when the two proteins are co-expressed *in vitro*. From a parallel clinical study we have identified non-synonymous mutations and intronic deletions in the RGS9 gene that are enriched in patients with schizophrenia and Parkinson's disease and that these RGS9 gene variations produce alterations in RGS9-2 cellular functions.
Conclusion: RGS9-2 is an important molecular participant in the abnormal modifications of the basal ganglia movement controlling circuits that lead to DID.

A24

Early Embryonic Exposure to Aroclor 1254 Targets Hsc70 Expression Resulting in Altered Central Nervous System Development.

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Problem: Polychlorinated biphenyls (PCBs) are persistent environmental contaminants that have neurological effects in children exposed in utero.
Methods: To define neuronally-linked molecular targets during early development, zebrafish embryos were exposed to Aroclor 1254, a mixture of PCB congeners that are common environmental contaminants.
Results: PCB exposure led to a reduction in the area of the ventro-rostral cluster at 24 hpf. PCB exposure resulted in a decrease in the number of neuronal branches emanating from the six major neurons originating from the RoL1 cluster at 24 hpf. Contrary to these findings, neuronal branching increased in PCB treated embryos at 48, 72, and 96 hpf. The supraoptic neuron arising from the trigeminal ganglion at 48 hpf had twice the number of branches. The serotonergic neurons in the developing diencephalon were also affected, showing a 34% reduction in serotonin concentration in the neurons. The size of the labeled neurons was not significantly different indicating that the development of the neurons was not affected, only the production of serotonin within the neurons. Microarray analysis of the zebrafish genome revealed consistent changes in 38 genes, 55% (21) of which are neuronally related. Heat shock protein 70 cognate (Hsc70) showed a consistent 50% reduction. The reduction in Hsc70 expression was confirmed by real-time PCR, revealing a consistent 30% reduction in expression in PCB treated embryos.
Conclusions: The PCB-induced structural and biochemical changes in the developing CNS may lead to alterations in the function of the affected regions.
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A25

BIOENGINEERED SKIN AND ITS DIFFERENTIAL KERATIN EXPRESSION DURING EPIBOLY AND HEALING

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Numerous studies have examined the expression of various keratin intermediate filaments in the process of keratinocyte migration in wound healing. We have recently demonstrated the feasibility of using epiboly in a bilayered bioengineered living skin construct (LSC) as an *in vitro* model to study keratinocyte migration. Here, we determined keratin expression in this epiboly model to better understand the keratin expression patterns during epidermal migration. Our results in this model support the expression pattern previously reported *in vivo.* We found that K1 and K10 were fully expressed in the epidermis of the fully epibolized surface. K1 was also faintly present in the migrating edge, while K10 was absent. K16 and K6 were evident in normal skin and the epibolized area; with K6 markedly present in the migrating edge. Importantly, K17 was distinctly limited to the epibolized surface and the migrating edge. Differential expression of keratins in this epiboly model reflects *in vivo* studies and suggests keratin specificity in the processes of migration and differentiation of new epidermis. Therefore, these findings provide further and important validity for the study of epithelialization in this epiboly model of a bilayered organotypic culture.

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A26

Recruitment of stem/progenitor cells to orthotopic malignant mesothelioma spheroids

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Problem: Malignant mesothelioma is an aggressive cancer of the mesothelial lining causally linked to asbestos exposure and is highly resistant to current therapies. We hypothesize a stem/progenitor cell population can be recruited to the tumor and contribute to tumor cell growth and progression.
Methods: Using an orthotopic murine tumor spheroid model shown to recapitulate human diffuse malignant mesothelioma, we identified a stem cell antigen-1 (Sca-1) positive cell population recruited to tumor spheroids *in vivo*. The Sca-1+ cell population was characterized by immunofluorescence staining and flow cytometry. Candidate chemokines involved in recruitment of this stem/progenitor cell population were identified with SuperArray™, and confirmed with transwell migration assays. To test whether this recruited cell population contributes to tumor cell proliferation, malignant mesothelioma cells were grown in conditioned media from *in vitro* cultures of this stem/progenitor cell population and tumor cell proliferation was assessed.
Results: The Sca-1+ cell population is comprised of T lymphocytes, hematopoietic stem cells and mesenchymal stem cells. The tumor microenvironment expresses the chemokine SDF-1 /Cxcl12 and the growth factor PDGF, both involved in recruitment of these cell types. Involvement of both chemotactic axes was confirmed when transwell migration of Sca-1+ cells to lavage fluid was abrogated by treatment of Sca-1+ cells with small molecule inhibitors of the receptors Cxcr4 and PDGFR respectively. Once recruited, this stem/progenitor cell population secretes factors that increase tumor cell proliferation.
Conclusions: A stem/progenitor cell population is recruited to malignant mesothelioma spheroids, secretes factors that promote tumor cell growth and is a potentially novel therapeutic target.

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A27

MARKERS OF FETAL NICOTINE EXPOSURE AND NEONATAL NEUROBEHAVIOR: THE NATIONAL COLLABORATIVE PERINATAL PROJECT

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Problem: We previously showed effects of self-reported smoking during pregnancy on infant neurobehavior. Here, we investigate dose response associations between two markers of fetal nicotine exposure (maternal cotinine; maternal nicotine metabolism) and infant neurobehavior.

Methods: Participants were 161 mothers (52% active smokers) and their healthy infants. Cigarettes per day (CPD), serum cotinine (COT) and serum trans-3'-hydroxycotinine (3HC) were assessed at 31-36 weeks gestation. Infant neurobehavior was assessed at days 0-3 using irritability and muscle tone subscales derived from the Graham-Rosenblith Behavioral Exam.

Results: After inclusion of significant covariates, multiple linear and logistic regression analyses revealed significant associations between maternal COT and infant irritability and muscle tone. Among infants of active smokers (COT>=10ng/mL; n=78), we explored the role of nicotine metabolism (quantified as 3HC/COT) in the associations between maternal smoking and infant neurobehavior. Continuous associations between nicotine metabolism and infant irritability trended toward significance, with greater irritability associated with slower nicotine metabolism. While no continuous associations between nicotine metabolism and infant muscle tone emerged, we found a significant interaction between CPD and nicotine metabolism in predicting infant muscle tone. Infants of moderate smokers (<20 CPD) with slower nicotine metabolism were similar to infants of heavy smokers (20+ CPD) and more hypertonic than infants of moderate smokers with faster nicotine metabolism.

Conclusions: Results reveal evidence for dose response effects of maternal nicotine and influences of maternal nicotine metabolism on infant neurobehavior. Fetal nicotine exposure mediates effects of maternal smoking on infant neurobehavior, and has implications for understanding links to long-term behavioral dysregulation.

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A28

COMPUTER OPTIMIZED SCREW PLACEMENT FOR VOLAR PERCUTANEOUS SCAPHOID FRACTURE FIXATION

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Purpose: Advances in surgical techniques have allowed for minimally invasive percutaneous fixation of acute scaphoid fractures. Previous studies have shown advantages to central screw placement; however such placement is often surgically inaccessible. This purpose of this study was to develop a means of calculating an ideal screw axis that is accessible via the volar approach.
Methods: For safe screw placement, the central screw axis must be contained within a three-dimensional “safe zone” created 3.325mm from the cortical surface of three-dimensional computer models of the right scaphoid from 10 subjects. Two methods for screw axis calculation were compared: Maximum screw length (MSL) within the safe zone and best-fitting a cylinder (CYL) to the safe zone. Resultant screw axes were compared for accessibility via the volar approach, resultant screw length, and the location of the screw axis relative to the volar tubercle and the scaphoid centroid.
Results: All of the MSL axes were accessible via the volar approach, compared to none of the CYL axes. The average MSL axes were 11% longer and passed significantly closer to the volar tubercle than the CYL axes. The MSL axes passed 1.6mm volar and proximal to the bone centroid, which was significantly farther than the CYL axes (0.4mm).
Conclusions: The MSL method provides a repeatable means for calculating an ideal screw axis, and enables a quantitative method to evaluate screw placement and provide a target during computer guided surgery.

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A29

Crystal Structure of TIGAR and Implications in Tumor Cell Metabolism Regulation

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Problem: The tumor-suppressor protein p53 inhibits the growth of malignant cells by inducing apoptosis and cell-cycle arrest, maintaining the genomic stability. TIGAR, TP53-induced glycolysis and apoptosis regulator, is a newly found bona fide target gene product of p53. TIGAR showed fructose-2,6-bisphosphatase activities in a previous study. By lowering the level of fructose-2.6-bisphosphate in the cell, TIGAR inhibits glycolysis and the generation of reactive oxygen species, thus protecting cells against ROS-related damage and blocking the energy supply in cancer cells.
Methods: We determined the structure of the human TIGAR homolog from *Danio rerio* at 2Å resolution by x-ray crystallography.
Results: The overall structure of TIGAR is similar to structures of other histidine phosphatases. Briefly, the TIGAR structure consists of a central domain and a small excursion composed of two α-helices and one β-sheet. The central domain is showing an α/β/α sandwich architecture in which central β-sheets are flanked by α-helices on both sides. The structure of TIGAR also reveal an unexpected dimerization interface, in which one β-strand from each molecule stack together in a anti-parallel manner, two potassium ions coordinated with valine 195 and proline 203 form the main interactions between two molecules, an intermolecular disulfide bond between cystine 201 from both molecules further stabilizes this interface. Dock 6 program predicts that fructose-2,6-bisphosphate can be well fitted in the substrate binding site, with both phosphate of fructose-2,6-bisphosphate coordinated with stabilizing amino acids.
Conclusion: These observations support the previous claim that TIGAR is a fructose-2.6-bisphosphatase.

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A30

Role of Natriuretic Peptide Receptors in Pulmonary Circulation

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Abstract:
Atrial and C-type natriuretic peptides (ANP, CNP) are known vasodilators. ANP binds to natriuretic peptide receptor (NPR) -A and –C. CNP binds to NPR-B and -C. The role of NPR-C in natriuretic peptide (NP) mediated pulmonary vasodilation remains unknown. The role of endothelium in mediating vasoactive effects of NP is controversial. We sought to define the role of NPR-C and to explore the role of the endothelium in NP mediated vasodilation in the pulmonary vasculature.
Methods: Using isolated perfused lungs from Sprague-Dawley rats we monitored pulmonary artery (PA) pressures upon exposure to increasing doses of Angiotensin II in the presence of vehicle or ANP, CNP, or cANF, an NPR-C agonist. Additionally, in isolated PA rings constricted with phenylephrine, concentration dependent relaxations were measured in response to NPs in endothelium intact and denuded vessels, or in presence of various inhibitors vs. vehicle.
Results: ANP and CNP but not cANF significantly attenuates the vasoconstrictive properties of Angiotensin II in isolated perfused lung. cANF demonstrates no vasodilatory effect in pre-constricted PA rings. ANP and CNP vasodilated the PA rings, but only CNP demonstrates endothelium dependent vasodilation. This was abolished by pretreatment with l-NAME a NO synthase inhibitor and iberiotoxin, a large-conductance Ca^{+2} activated K^{+} channel blocker (BK_{Ca}). 18 α-glycyrrhetinic acid (18α-GA), a myoendothelial gap junction inhibitor, and indomethacin, a cyclooxygenase inhibitor, had no effect on endothelium dependent vasodilation.
Conclusions: NPR-C plays limited role in NP mediated pulmonary vasodilation. The endothelium dependent effect of CNP is mediated by NO and BK_{Ca} channels.

A31

EPIGENETICS AND PLACENTAL GENE PROGRAMMING

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Problem: The placenta expresses key neuroendocrine genes which govern the intrauterine environment. Chromatin remodeling or DNA methylation at specific gene promoters may be a key mechanism of environmentally induced gene expression in the placenta. This altered gene expression may be associated with later effects on health and disease.
Methods: We examined the changes in global DNA methylation and expression of the norepinephrine transporter gene (NET) and the steroid metabolizing enzyme 11βHSD2 in adverse and low risk control placentas. The cytosine extension assay was used to detect changes in methylation in genomic DNA and in CpG islands corresponding to promoter regions. Gene expression was examined by Northern analysis. Promoter methylation was examined with methylation sensitive PCR.
Results: Global methylation was increased in adverse pregnancies, particularly in promoter regions. Placental expression of both NET and 11β-HSD2 was reduced following intrauterine exposure to nicotine, cocaine or other adverse events. Hypermethylation of the 11βHSD2 promoter was increased in drug exposed placentas compared to controls.
Conclusions: Down regulation of expression may be secondary to epigenetic changes in the NET and 11β-HSD2 promoters. This gene-environment interaction and permanent alteration in methylation state suggests an important epigenetic mechanism for long term placental gene programming as a consequence of an adverse intrauterine environment. These studies will provide important mechanistic links between prenatal stress and early phenotypic clues to the fetal origins of adult disease.

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A32

Core Research Laboratories (CRL)
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Core Research Laboratories (CRL) is a network of centralized facilities which offers a broad spectrum of technological services to the research community at large on a fee-for-service basis. The technologies provided include histology, flow cytometry, electron microscopy and digital imaging.

The Transmission Electron Microscopy (TEM) Laboratory offers a wide range of ultrastructural technologies including negative staining; standard fixation, embedding and sectioning protocols; pre and post embedding cytochemical and immunoloigcal localization techniques using periodase and colloidal gold markers; and the use of various acrylic resins (to improve retention of biological reactivity

The Fluorescence Activated Cytometry/ Sorting (FACS) Lab provides rapid multi-parameter analysis and sorting of cells, and other entities. Measurable parameters for high-speed analysis include: cell or particle size; cytoplasmic granularity; and fluorescence emission in four different fluorescent bands

The Histology Laboratory provides histological and cytological services including: processing; embedding; sectioning (fixed and fresh frozen, paraffin, methacrylate, et. al); staining-conventional, immunological with fluorescent or peroxidase-tagged probes, cytological procedures-cytospins, cell pellets, cell cultures, smears, et. al.

The Digital Imaging Laboratory offers a variety of advanced digital technologies for image acquisition, processing, analysis, and storage Including Live-cell Confocal Microscopy.

The Division of Core Research Laboratories is located on the RIH in the Coro (West) Building, 1Hoppin Street, Suite 2-410 Please visit our web site at: http://www.lifespan.org/reslab

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A33

IN VIVO METHOD FOR TRACKING 3D KINEMATICS OF THE HEALTHY AND ANTERIOR CRUCIATE LIGAMENT TRANSECTED GOAT STIFLE JOINTS: A PRELIMINARY STUDY

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The objective of this study was to introduce a novel method for tracking 3D kinematics of the healthy and anterior cruciate ligament (ACL) transected goat stifle joints. Sham and ACL-transection surgeries were performed on two goats (1 sham, 1 ACL-transection) with tantalum bone markers inserted into the tibia and femur. Gait was captured on a treadmill using a high-speed biplanar fluoroscopy system. Bone markers were digitized to extract their 3D coordinates over the entire gait cycle. A method for defining a clinically relevant anatomical coordinate system (ACS) was developed based on bone features present on 3D bone models from computed tomography (CT) scans of the two goat stifle joints. The defined ACS was linked, rotoscoped, and animated to extract meaningful rotational and translational gait kinematics. Rotational ranges of motion (ROM) measurements were calculated as the difference between the maximum and minimum peaks of flexion/extension (FE), ab/adduction (AB/ADD), and internal/external (IE) rotation. The largest ROM in the goat knee joint was FE (sham 37.7° ± 2.43°, ACL-transected 32.7° ± 3.30°), followed by IE rotation (sham 18.1° ± 4.41°, ACL-transected 22.5° ± 3.33°), and then AB/ADD (sham 4.76° ± 1.29°, ACL-transected 8.37° ± 2.21°). These data suggest that the ACL-transected goat's stifle joint had a more difficult time achieving full flexion and was more unstable than the sham goat's stifle joint. Furthermore, these data indicate the ability to and repeatability of tracking 3D kinematics *in vivo*.

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A34

Polymeric Beads Amplification Strategy for Sensitive Electrochemical Detection of Matrix Metalloproteinase-3, a Cancer Biomarker Protein in serum

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Problem: Despite recent advances in treatment, cancer still remains a major leading cause of death in the world. Rapid, specific *early detection* of cancer biomarkers proteins in serum is the only hope to change this fact. Such sensitive detection schemes are expected to greatly improve patient prognoses, treatment success, and even lead to cancer prevention. The broad long-term goals are to develop nanomaterial-based arrays to measure collections of early cancer biomarker proteins for specific forms of cancer.
Method: Polymeric beads amplification strategy was used for sensitive Electrochemical detection of Matrix Metalloproteinase-3 (MMP-3), a cancer biomarker protein in serum. The detection of human MMP-3 is based on a sandwich immunoassay captured on single-wall carbon nanotubes arrays (SWNT) in connection with catalytic reactions involving horseradish peroxidase tracer secondary antibody. The SWNT were characterized using Atomic Force microscopy (AFM) and Resonance Raman spectroscopy.
Results: AFM confirmed bundles of vertically aligned SWNT with average vertical height ~ 20-40 nm. The performance of the electrochemical immunoassay was evaluated and optimized. The non-amplified approach provided a detection limit of 4.0 x 10-10 g mL-1 (5.0 pM), which corresponds to 50 amol of MMP-3 in 10 L calf thymus serum samples while the novel polymeric beads amplification strategy gave a detection limit of 10.0 x 10-12 g mL-1 (125 fM) corresponding to 1250 zepto mol of MMP-3 in 10 L serum sample.
Conclusions: This immunosensor based on SWNT arrays offers great promise for a rapid, simple, cost-effective method for clinical screening of cancer biomarkers and point-of-care diagnosis.

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A35

INHIBITION OF *CANDIDA ALBICANS* ADHESION BY RECOMBINANT HUMAN ANTIBODY SINGLE-CHAIN VARIABLE FRAGMENT SPECIFIC FOR ALS3p

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Problem: *Candida albicans* causes severe infections in the immunocompromised. The transition of *C. albicans* from yeast to hyphal growth morphology has been associated with its virulence. We have previously reported isolation via phage display of human antibody fragments (scFv) specific to the hyphal form of this organism. We identified the *C. albicans* adhesin, Als3p, as a potential cognate antigen for these scFv based on similarity of the binding pattern of the scFv to the distribution of this protein on the hyphal surface.
Methods: Immunofluorescence assays were conducted to determine binding patterns of scFv to strain 1843, containing a homozygous deletion of *ALS3*. To determine if scFv3 could interfere with Als3p function, a fluorescence based adhesion assay in 96-well format was developed using both human epithelial (FaDu) and endothelial (HUVEC) cells as target.
Results: Although all scFv bind avidly to wild-type, scFv3 showed no detectable binding to 1843 while binding to the *ALS3* reintegrant strain, 2322, was preserved. Other scFv (scFv5 and scFv12) retained binding to 1843, but with an obviously altered pattern. The previously reported adhesion defect of strain 1843 relative to wild-type was confirmed. Further, treatment of wild-type *C. albicans* with scFv3 reduced adhesion of the fungus to both epithelial and endothelial cells to levels comparable to the *als3*Δ/*als3*Δ mutant.
Conclusions: Phage display is a viable method to isolate human scFv specific to an antigen implicated in virulence of *C. albicans*, and they have a role in interference with adhesion to human cells.

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A36

The Role of Complement Activation in Preeclampsia

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Objective: Preeclampsia contributes significantly to maternal and neonatal mortality and morbidity. Delineation of a mechanistic pathway(s) for preeclampsia has remained a complex issue because of its heterogeneous etiology. We hypothesize that serum from preeclampsia patients can serve as a blueprint for the pathology of preeclampsia. Here we address an association between complement activation and preeclampsia.
Methods: Serum samples from normal pregnancy and preeclampsia patients were collected in a gestational age-matched manner. To evaluate the serum-induced cytotoxic effects, we employed TCL-1 cells (third trimester extravillous trophoblast cell line), HTR-8 cells (first trimester extravillous trophoblast cell line), and freshly isolated term primary trophoblasts. To evaluate the nature of the cytotoxic activity in preeclampsia serum, these samples were inactivated using several approaches. Analysis of complement regulatory proteins, CD55, CD59 and CD46, on trophoblasts was performed by FACS and immunohistochemistry (IHC). Terminal complement complex (TCC) deposition on TCL-1 and HTR-8 cells was evaluated by immunofluorescence.
Results: Forty percent of the preeclampsia serum samples tested caused apoptosis in TCL-1, but not in HTR-8 or primary trophoblasts. Heat inactivation, heparin treatment or treatment with C5 and C9 neutralizing antibodies prevented PE serum-induced TCC deposition on and apoptosis of TCL-1 cells. TCC deposition was not observed on HTR-8 trophoblasts. IHC showed extensive CD55 staining in normal, but not preeclampsia placental tissue. FACS analysis revealed that HTR-8 and primary trophoblasts expressed higher levels of CD55 and CD59 compared to TCL-1.
Conclusions: Complement activation appears to be one of the mechanisms for placental deficiencies associated with preeclampsia.

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A37

COMBINED TREATMENT OF NON-HEALING WOUNDS WITH BIOENGINEERED SKIN AND MESENCHYMAL STEM CELLS

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PURPOSE: Systemic sclerosis (scleroderma) ulcers are difficult to treat. We have tested the safety and feasibility of topical application of autologous bone marrow-derived cultured mesenchymal stem cells (MSC).
METHODS: Three patients with scleroderma ulcers were treated. Following bone marrow aspiration, autologous MSC were established and expanded in culture. Flow cytometry, immunohistochemistry, and functional studies were used to determine the phenotype of mesenchymal stem cells.
RESULTS: No safety problems surfaced with bone marrow aspiration and the application of autologous MSC to the wounds. On immunohistochemistry and flow cytometry, cultured MSC showed the following stable surface markers characteristics: CD29+, CD44+, CD90+, CD105+, CD166+, and CD34- (hematopoietic cell marker) and CD45-. In vitro studies showed that the cultured cells were capable of differentiation into bone, cartilage, and adipose tissue. A fibrin spray system, which takes advantage of the polymerization of cells-containing fibrinogen when mixed with thrombin, was used to deliver the cultured mesenchymal stem cells to the wound in a fine spray. The fibrin could also be delivered by dripping it over the wound. Pitting scars were treated as well, to determine a possible positive effect and to ensure that no ulceration would result from the experimental treatment. Up to 5 applications of MSC were performed. The treatment resulted in dramatic wound healing. The pitting scars also seemed to improve.
CONCLUSIONS: We have developed novel techniques for culturing and delivering autologous bone marrow-derived MSC to scleroderma ulcers. We have characterized the phenotype of these cells both in vitro and in vivo. The outcome appears promising.

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A38

GENERATION AND CHARACTERIZATION OF A NOVEL, CARDIAC-SPECIFIC, INDUCIBLE RGS2 TRANSGENIC MOUSE MODEL TO MODULATE G_q-MEDIATED CARDIAC HYPERTROPHY

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Problem: Hypertrophy is a response of the heart to increased workload that often results in heart failure. Receptor signaling via heterotrimeric G_q proteins leads to hypertrophy *in vitro* and *in vivo*. RGS proteins are negative Regulators of G-protein Signaling. Among them, only RGS2 is a selective negative regulator of G_q signaling and hypertrophy in cardiomyocytes. We hypothesize that RGS2 exerts similar effects *in vivo*, which could be beneficial to hearts subjected to increased workload. Our initial objective was to generate a mouse model suitable to test whether RGS2 overexpression can regulate G_q signaling and modulate the development and/or reversal of hypertrophy *in vivo*.
Methods: We generated transgenic mice with cardiac-specific, inducible RGS2 expression (TG) using a doxycycline (Dox)-responsive α-myosin heavy chain promoter. Real-time PCR and Western blots were used to characterize tissue specificity, subcellular localization and Dox-dependence of RGS2. Phospholipase Cβ (PLCβ) activity in cardiac tissue measured G_q signaling.
Results: RGS2 expression was pronounced and restricted to the myocardium in TG. It was dose-dependently regulated by Dox (i.e., suppressed by Dox within days and re-expressed within 1 month after Dox withdrawal). G_q-coupled receptor-stimulated PLCβ activation was diminished in RGS2-expressing atria and ventricles. Other major RGS proteins were not changed in TG hearts.
Conclusions: Proper regulation and function of RGS2 with no compensatory changes in other RGS proteins suggest that this transgenic model can be used to time RGS2 expression in mice such that the role of RGS2 as regulator of G_q signaling and hypertrophy can now be assessed *in vivo.*

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A39

NUTRIENT REGULATION OF CHONDROCYTE PROLIFERATION AND DIFFERENTIATION

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Problem: Poor nutrition leads to poor linear growth. The objective of this study was to examine if restricted leucine availability could inhibit chondrocyte proliferation and differentiation at the growth plate, focusing on the effect of leucine on the nutrient-sensing kinase mTOR (mammalian Target of Rapamycin) and alternate pathway, GCN2.
Methods: An *in vitro* fetal (E19) rat metatarsal model was utilized with explant culture in condition-specific α-MEM media for 72 hours. Insulin (1600 nM) was a growth-enhancing factor in all media. Experimental conditions included varied leucine concentrations (100% or 1.6 mM, 25%, 10%, 5%) +/- known mTOR inhibitor rapamycin (50 nM). Metatarsal lengths were measured daily. Growth plates were examined on H&E sections. BrdU (indicating cell proliferation), ribosomal protein S6 phosphorylation (PS6, readout of mTOR activity), and eIF2α phosphorylation were measured. Similar studies were performed in ATDC5 cells.
Results: Leucine restriction produced a dose-dependent inhibitory effect on metatarsal explant growth, with decreasing lengths (P values <0.001). This was accounted for by inhibition of proliferation as measured by BrdU (P <0.001) and decreased length of the hypertrophic zone (P<0.01). Rapamycin-exposed metatarsals showed limited growth inhibition (P values <0.05) with complete PS6 inhibition; however, S6 phosphorylation was only partially inhibited by leucine restriction (P<0.01). In ATDC5 cells, phosphorylation of eIF2α was increased in leucine restriction.
Conclusions: Bone growth was sensitive to leucine deprivation. Both chondrocyte proliferation and hypertrophy were inhibited. This was accompanied by only partial inhibition of mTOR signaling, suggesting a role for an alternate amino acid-responsive pathway such as GCN2 kinase.

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A40

ELONGATION OF THE EXTRINSIC RADIOCARPAL LIGAMENTS

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Wrist stability is dependent on both the bony articulations and the ligamentous structures of the carpus. However, little is known about the in vivo behavior of the carpal ligaments as the wrist moves through its full range of motion. A computational ligament model is proposed. The model uses carefully defined ligament insertion regions in combination with 3-D surface models of the carpal bones and an algorithm that constrains the ligament fibers to wrap around the bones as the wrist moves through it's full range of motion. The major extrinsic radiocarpal ligaments, the long and short radiolunate (LRL, SRL), the radioscaphocapitate (RSC), and the dorsal radiocarpal (DRC) ligaments, were modeled in vivo for 16 healthy subjects (32 wrists). The hypothesis that the ligament fibers in the model would vary as a function of wrist flexion / extension was tested using linear regression. The results show that with the exception of the SRL, the radiocarpal ligaments varied significantly ($P < 0.01$) as the wrist moved in flexion and extension. The opposing behavior of the DRC and the volar ligaments is consistent with the volar ligaments acting as stabilizers during wrist extension and the dorsal ligaments acting as stabilizers during wrist flexion.

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A41

Membrane insertion/folding of pHLIP peptide

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Problem: The pHLIP peptide - pH Low Insertion Peptide serves as a model system for peptide insertion/folding across a lipid bilayer. It has three general states: (I) soluble in water (II) bound to the surface of a lipid bilayer being unstructured, and (III) inserted across the bilayer as an α-helix.
Methods: We used various biophysical techniques including fluorescence spectroscopy and microscopy, isothermal titration calorimetry and circular dichroism to study pHLIP interaction with the lipid bilayer of liposomes.
Results: Our results indicated that:

1) pHLIP is monomeric in all three states;
2) the insertion of pHLIP in lipid bilayer is unidirectional: C-terminus goes inside cell and the N-terminus stays outside. A variety of cargo molecules attached to the C-terminus of pHLIP *via* cleavable S-S bond could be translocated and released in cytoplasm in the environment of low extracellular pH.
3) the molecular mechanism of pHLIP insertion across the lipid bilayer is associated with the protonation of Asp residues induced by low pH, which triggers by peptide hydrophobicity and membrane insertion/folding.
4) the Gibbs free energy of pHLIP binding to the surface of lipid bilayer is about -7 kcal/mol near neutral pH at 37C and the insertion free energy is nearly -2 kcal/mol - the amount of energy that might be used to move cargo molecules across a membrane.

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Conclusions: Our studies advance understanding of fundamental principles of insertion and folding of proteins in membrane and use these principles in Medicine for diseased tissue targeting and drugs translocation through the membrane inside cell.

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A42

PFaST : Protein Fluorescence and Structural Toolkit

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The major goal in the application of tryptophan fluorescence spectroscopy is to interpret fluorescence properties in terms of structural parameters and to predict of the structural changes in the protein. We have developed methods for the mathematical analysis of fluorescence spectra of multitryptophan proteins aimed at revealing the spectral components of individual tryptophan or clusters of tryptophan residues located close to each other (Burstein et al., 2001, *Biophys J*., 81, 1699-1709; Reshetnyak and Burstein, 2001, *Biophys. J*., 81, 1710-1734). Also, we have created an algorithm for the structural analysis of the tryptophan environment in 3D atomic structures of proteins from PDB (Reshetnyak et al, 2001, *Biophys. J*., 81, 1735-1758). The successful design of the methods of spectral and structural analysis opened an opportunity for establishing a relationship between the spectral and structural properties of a protein. We have integrated the developed software modules, introduced new programs for the assignment of tryptophan residues to spectral-structural classes, and created a web-based toolkit PFAST: Protein Fluorescence and Structural Toolkit (Shen et al., 2008, *Proteins*, 71, 1744-1754). PFAST contains 3 modules: 1) FCAT - fluorescence-correlation analysis tool, which decomposes protein fluorescence spectra and assigns spectral components to one of five previously established spectral-structural classes. 2) SCAT - structural-correlation analysis tool for the calculation of the structural parameters of the environment of tryptophan residues from the atomic structures of the proteins from the PDB, and for the assignment of tryptophan residues to one of five spectral-structural classes. 3) The last module is a PFAST database.

A43

New technology for translocation of molecules through the membrane into cells

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Problem: Majority of drugs are membrane-permeable molecules affecting cells not only in diseased tissue, but also in healthy tissue increasing side effects and reducing efficacy of treatment. We are developing a novel technology for selective translocation of membrane –impermeable molecules. It is based on action of water-soluble membrane peptide, pHLIP (pH (Low) Insertion Peptdie), which has ability to insert in cell membrane only at slightly acidic environment, which is characteristics for various pathological states, such as cancer, inflammation, stroke and others.
Method: Cell–impermeable cargo molecule was attached to the C-terminus of pHLIP using a disulfide bond which, once translocated, was easily cleaved inside the cell releasing the cargo into the cytoplasm. Various types of cancer cells were treated with fluorescently labeled cargo and pHLIP-cargo at low (pH6.5) or normal (pH7.4) pHs. Translocation was monitored by fluorescence microscopy and fluorescence activated cell sorting. Standard MTS assay was performed to study cell proliferation.
Results: pHLIP peptide was able to translocate cell-impermeable cargo molecules through the membrane in cytoplasm in a pH-dependent manner. Among translocated molecules are fluorescent dyes, cyclic peptide phalloidin (mushroom toxin) conjugated to rhodamine and gene regulation agent, peptide nucleic acid (PNA). Phalloidin-rhodomine stabilized cell cytoskeleton and led to the formation of multinucleated cells. PNA induced expression of protein.
Conclusion: The unique properties of pHLIP peptide open opportunities for the delivery and translocation of a variety of biopharmaceuticals (not affecting normal cells in healthy tissue) into cells. This would dramatically enhance therapeutic efficacy and reduce side effects.

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A44

Effects of Single and Multiple Courses of Maternal Dexamethasone Treatment on Tight Junction Proteins Expression in the Cerebellum of Fetal Sheep

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Background: The tight junctions (TJ) are the main structures responsible for the properties of the blood-brain barrier (BBB). We have shown that antenatal corticosteroids reduce BBB permeability in the brain regions of ovine fetuses. Although single courses of antenatal corticosteroids are widely used to accelerate fetal maturation, multiple courses are sometimes given to women at recurrent risk for premature delivery. The effects of single and multiple courses of corticosteroids on TJ proteins have not been examined in different regions of the fetal brain.
Objective: To examine the effect of single and multiple courses of dexamethasone (DEX) on TJ protein expression in the cerebellum (CL) of fetal sheep.
Design/Methods: Chronically catheterized fetuses at 70% of gestation were examined after a single course of four placebo (PL, n=6) or 6 mg DEX (n=6) injections were given every 12 h for 48 h to ewes, or the same PL (n=6) or DEX (n=6) course was given once a wk for 5 wks. CL was snap frozen 18 h after the last PL or DEX dose at 107 days of gestation. Occludin (Occl), claudin-1(CL-1), claudin-5 (CL-5), and zonula occludens (ZO) 1 and 2 protein expression were determined by Western blot analysis, densitometry performed and results expressed as ratio to the internal control samples.
Results: Both single and multiple courses of maternal DEX resulted in a significant increases in Occl and CL-1 protein expression, but not any of the other TJ proteins that we examined in the cerebellum of fetal sheep (Fig; M±SEM; * $P<0.05$).
Conclusions: Based on our previous findings in cerebral cortex (Abstract # 750304), we conclude that corticosteroids may have different effects on the regulation of TJ proteins in different regions of the brain in the ovine fetus. Funded R01-HD34618

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A45

Title: Effects of Single and Multiple Courses of Maternal Dexamethasone Treatment on Tight Junction Protein Expression in the Cerebral Cortex of Fetal Sheep

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Background: The blood-brain barrier (BBB) is composed of endothelial cells connected by intercellular tight junctions (TJ). TJs are complex molecular structures composed of transmembrane and cytoplasmic proteins. Antenatal steroids reduce BBB permeability in the fetus. Although a single course of steroids is used to accelerate fetal maturation, multiple courses are given to women at recurrent risk for premature delivery. The effects of single and multiple courses of steroids on TJ proteins have not been examined in fetal brain.
Objective: To examine the effect of single and multiple courses of dexamethasone (DEX) on TJ proteins in the cerebral cortices (CC) of fetal sheep.
Design/Methods: Fetuses at 70% of gestation were examined after a single course of four placebo (PL, n=6) or 6 mg DEX (n=6) injections were given 12 h apart for 48 h to ewes, or the same PL (n=6) or DEX (n=8) course given once a wk for 5 wks. CC were frozen 18 h after the last dose at 107 d of gestation. Occludin (Occl), claudin-1(CL-1), claudin-5 (CL-5), zonula occluden (ZO) 1 and 2 protein expression were determined by Western blot, densitometry performed and results expressed as ratios to internal control samples.
Results: A single course of maternal DEX resulted in increases in CL-5 expression, but not in the other TJ proteins that we measured (Fig; M±SEM; $P<0.05$). Multiple courses of DEX resulted in increases in Occl and ZO-1, but not CL-1, CL-5 or ZO-2 expression.
Conclusions: Our findings are the first to demonstrate that exogenous steroids regulate TJ proteins *in vivo* in the cerebral cortex. Single and multiple courses of antenatal corticosteroids have different affects on the regulation of specific tight junction proteins. NIH R01-HD34618.

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A46

MECHANICAL STRETCH PROMOTES FETAL TYPE II EPITHELIAL CELL DIFFERENTIATION VIA SHEDDING OF HB-EGF AND TGF-α LIGANDS

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Problem: Mechanical forces are critical for normal lung organogenesis. However, the mechanisms by which stretch promotes lung development are unknown. We hypothesized that mechanical stretch promotes fetal type II cell differentiation via release of membrane-anchored EGFR ligands.
Methods: E19 fetal type II cells were incubated with several recombinant EGFR ligands. Cell differentiation was assessed by surfactant protein B/C (SP-B/C) mRNA and protein levels. Analysis and quantification of ligands shedding were performed by measuring alkaline phosphatase (AP) activity in the supernatant after transfection with cDNA constructs encoding AP-EGFR ligand fusion proteins and exposure to different mechanical stretch protocols.
Results: Incubation of E19 cells with rHB-EGF or TGF-α increased SP-B/C mRNA and protein levels. Cyclic stretch released HB-EGF and TGF-α into the supernatant. This effect was negligible after 2.5% stretch and significantly increased with higher magnitudes of strain. Release of these ligands was most remarkable in E18-19 cells and minor in E20 cells. Mechanical stretch also increased HB-EGF and TGF-α mRNA and protein levels.
Conclusions: Mechanical stretch promotes type II cell differentiation via release of HB-EGF and TGF-α ligands. The magnitude of shedding depends on gestational age and strain protocols. These studies provide important mechanistic information potentially relevant to fetal lung development and also to mechanical ventilation-induced lung injury.

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A47

$GABA_A$ RECEPTOR LIGANDS ACTIVATE LIVER NUCLEAR RECEPTORS

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Problem: The constitutive androstane receptor (CAR) exists in an inactive state in the cytoplasm of hepatocytes and is activated by chemicals with which it does not directly interact. An indirect (non-ligand) activator of CAR is the sedative phenobarbital (PB), which causes CAR to translocate to the cell nucleus. PB and other transcriptional activators of CAR-target gene CYP2B6 are also ligands of gamma amino butyric acid A receptors ($GABA_AR$), and there are at least sixteen different $GABA_AR$ genes. We hypothesize that $GABA_AR$ are involved in the upstream activation of CAR. The objective of this study was to demonstrate that specific subunits of $GABA_A$ receptor are present in the liver and necessary for CAR activation.
Methods: Reverse transcription-polymerase chain reaction (RT-PCR) assays and western blot analyses of $GABA_A$ receptor mRNA and protein levels were utilized to measure the expression of receptor subunits. $GABA_AR$ ligands were screened for activation of CAR target gene CYP2B6 and HuH7 cell line was transiently transfected with CAR expression plasmid in order to measure CAR-driven luciferase reporter activity in response to treatments from a GABAergics chemical library.
Results: $GABA_A$ receptors are differentially expressed in HuH7 cells versus primary human hepatocytes. Multiple ligands of $GABA_A$ receptors are direct (ligand) and indirect (non-ligand) activators of CAR.
Conclusions: Indirect activation of CAR by neuroactive pharmacological agents may involve upstream perturbation of hepatocyte $GABA_A$ receptors.

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A48

STRESS RESPONSE AND THE ADOLESCENT TRANSITION: PERFORMANCE VERSUS SOCIAL REJECTION STRESSORS

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Problem: The adolescent transition is associated with dramatic increases in depression/psychiatric disorders; however, little is known about normative variation in stress response over this transition. This study examined neuroendocrine and cardiovascular responses to performance and peer rejection stressors over the adolescent transition in a normative sample.
Methods: Participants were 82 healthy children (ages 7-12 years, Tanner I-III, *n*=39, 22 females) and adolescents (ages 13-17 years, Tanner IV-V, *n*=43, 20 females) recruited through community postings. Following a habituation session, participants completed a performance (public speaking, mental arithmetic, mirror tracing) or peer rejection (exclusion challenges) stress session. Salivary cortisol, alpha amylase (*s*AA), systolic and diastolic blood pressure (SBP, DBP), and heart rate (HR) were measured throughout.
Results: We found consistently increased physiological responses to developmentally-relevant laboratory stressors across adolescence, with increasing development associated with increased HPA, SNS, and cardiovascular stress responses. Developmental influences on cortisol and DBP stress response were most pronounced in the context of the performance stressors, while developmental influences on *s*AA and SBP were evident primarily in response to the peer rejection stressor.
Conclusions: Heightened physiological stress responses in typical adolescents may facilitate adaptation to new challenges of adolescence and adulthood. In high-risk adolescents, this normative shift may tip the balance toward stress response dysregulation associated with depression and other psychopathology. Specificity of physiological response by stressor type highlights the importance of a multi-system approach to the psychobiology of stress and may also have implications for understanding trajectories to psychopathology.

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A49

SHORT-TERM EFFECTS OF PRENATAL SMOKING EXPOSURE: POSSIBILITY OF INFANTS IN WITHDRAWAL?

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Problem: Relatively little attention has focused on effects of maternal smoking during pregnancy (MSDP) on neurobehavior in the neonatal period. In the present study, we present preliminary evidence for a neonatal abstinence syndrome following exposure to MSDP.
Methods: We examined behavioral and cortisol response to the NICU Network Neurobehavioral Scale (NNNS) in exposed and unexposed infants matched across socio-economic status, and maternal age and alcohol use in two independent studies. Study 1 focused on 56 healthy infants (27 exposed) 24-48 hours old. Study 2 included 56 healthy infants (28 exposed) ages 10-27 days old. Smoking during pregnancy was assessed using the Timeline Followback interview and verified with saliva cotinine.
Results: In Study 1 (24-48 hours), smoking-exposed infants were more excitable, hypertonic, required more handling, showed greater signs of stress/abstinence and significant alterations in cortisol response relative to unexposed infants. In study 2 (10-27 days), smoking-exposed infants showed greater need for handling, worse self regulation and greater excitability and arousal. No effects of MSDP emerged for signs of stress/abstinence or cortisol response.
Conclusions: We found significant effects of MSDP on neonatal neurobehavior. Effects differed based on whether infant assessments were completed during the early or later neonatal periods. That signs of abstinence, tone, and stress response patterns were evident only in the newborn period but not at 10-30 days suggests the possibility of a withdrawal syndrome in exposed infants. Patterns of irritability and difficulty self-soothing in exposed infants at 10-30 days may represent an early link to later emotional/behavioral dysregulation.

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A50

HDAC4 DOWN-REGULATES VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN CHONDROSARCOMA CELLS BY MODULATING RUNX2 EXPRESSION

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Introduction: Angiogenesis is critical for tumor growth and metastasis. We tested the hypothesis that HDAC4/RUNX2/VEGF gene expression is dysregulated and that decreased HDAC4 expression accounts for some of the increased VEGF expression seen in chondrosarcoma.
Methods: Human chondrocytes and chondrosarcoma cell lines (CS) were transfected with expression constructs for HDAC4, Runx2, CBP, and/or VEGF promoter/pGL3 luciferase reporter plasmid or empty vector, and harvested for the following experiments.
Real-time RT-PCR for quantification of HDAC4, Runx2, and VEGF mRNA,
Luciferase Assay to measure VEGF promoter activity,
Western Blot Analysis with Anti-VEGF and anti-HDAC4 antibodies,
HDAC4 knock-down using HDAC4 siRNA, and
CHIP assay to assess binding of Runx2 to VEGF promoter.
Results: Endogenous HDAC4 mRNA level was 0.3 in CS whereas RUNX2 was increased 10 fold compared to chondrocytes.

CS transfected with HDAC4-pcDNA3 had ½ Runx2 mRNA and 1/3 VEGF mRNA;VEGF protein decreased. Runx2 transfection upregulated VEGF mRNA 2-fold.

To determine whether HDAC4 regulates VEGF directly or indirectly through RUNX2, a VEGF promoter activity experiment and CHIP was performed. Runx2 increased the activity of VEGF promoter; cotransfection of HDAC4 reversed the increase. When the activity of Runx2 was increased by p300/CBP, an acetylator, the activity of VEGF promoter increased more, but cotransfection of HDAC4 abrogated this increase. HDAC4 knock-down with siRNA had the opposite effect: VEGF mRNA increased 3.4 fold. CHIP showed Runx2 binds to the VEGF promoter.
Conclusion: Our results show that chondrosarcoma cell lines have reduced levels of HDAC4 resulting in increased Runx2 activity and VEGF mRNA and protein.

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A51

Polychlorinated biphenyls induce preterm birth by targeting aquaporin 1 and VEGF R2 at the maternal-fetal interface: Protective Role of IL-10

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Problem:Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants with estrogenic activity. Epidemiological and experimental evidence suggests their association with various health hazards. PCBs present a potential risk of disturbing the endocrine-immune-angiogenic axis, subsequently leading to adverse pregnancy outcomes. Gene-environment interactions may also suggest that a genetic stress may predispose the individuals to reproductive health risks. Here, we sought to examine the effect of PCB exposure on pregnancy outcome in IL-10 deficient and wild type mice.
Method:Pregnant IL-$10^{-/-}$ or congenic wild type mice were given i.p injections of Aroclor1254, a mixture of structurally different PCBs or vehicle at a dose of 500 ug/mouse from gestational day (gd) 4-12. Mice were euthanized on gd13, or allowed to deliver the pups. Tissues and fluids were collected for further analysis.
Results:Aroclor1254 caused preterm birth in IL-$10^{-/-}$ mice along with increased amniotic-fluid, reduced placental and fetal weight, poor litter size, and neurocognitive anomalies as demonstrated by defective Righting Reflexes in newborns. Pregnancy outcome in wild type counterparts was normal. The results strongly suggest that Aroclor1254 induced reduction in the expression of water channel Aquaporin1 (AQP1) and VEGF R2 in utero-placental tissue. Further, our *in vitro* experiments showed that Aroclor1254 disrupts angiogenic interactions between endothelial cells and trophoblasts, a process dependent on AQP1 and VEGF R2.
Conclusion:Taken together, our results suggest a role of IL-10 in protection against toxicant-induced pregnancy complications. AQP1 and angiogenesis are identified as novel targets of PCB action at the maternal-fetal interface. Supported by NIEHS SBRP award P42ES13660.

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A52

TLR3-Mediated Induction of Fetal Resorption and Preterm Birth: Involvement of Distinct Mechanisms in Wild Type and IL-10 Null Mice

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Problem: Systemic infections pose a threat to maternal fetal health, and lead to spontaneous abortion or preterm birth. A delicate balance of innate immunity is present at the maternal fetal interface often aiding in fetal development, yet this balance is negatively altered when infiltration of pathogens occurs via aberrant TLR ligation at the interface. We focus on activation of TLR3 with its known ligand, PolyI:C, in order to mimic the *in vivo* cascade of deviant immune events leading to fetal resorption (FR), and preterm birth (PT).
Methods: IL-10 null or WT mice were mated and vaginal plugs on gestational day 0 were separated for experimental treatment. Doses of PolyI:C were given I.P. on gd 6, or 14 to mimic infection in early or late stage pregnancy. Mice were harvested on gd 10-12, or 16-18 and their immune profiles from uterine tissues were assessed via flow cytometry or immunohistochemistry, and serum was collected for ELISA. Depleting antibodies for NK cells were utilized as were blocking antibodies to TNF .
Results: FR was induced in IL-10 null and WT mice at similar doses. Interestingly, WT mice were more sensitive in the PT scenario, succumbing to PT at lower doses than IL-10 null animals. Depletion of NK cells demonstrated that WT pregnancy was rescued, whereas IL-10 pregnancy was not. Flow cytometry demonstrated that CD3+ cells were important in the IL-10 null response to PolyI:C, but not in WT mice. Blocking of TNF was able to abrogate negative fetal outcomes in both mouse strains.
Conclusion: We demonstrate alternative cellular mechanisms leading to fetal rejection in response to PolyI:C in the IL-10 null and WT mice. However, both animal strains are susceptible to a common TNF - mediated axis.

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A53

Noncanonical Wnt11 Signaling Inhibits Hepatocellular Carcinoma Cell Proliferation.

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Background and Aims: Wnts are secreted lipoproteins that promote tumor formation when abnormally activated. Enhanced signaling through the canonical b-catenin pathway may be due to genetic alterations or up-regulation of Wnt/Frizzled-7 receptor (FZD7). These events are found in over 90% of HBV and HCV related hepatocellular carcinoma (HCC) and appear common early in the course of disease. In contrast, the biological role of noncanonical Wnt mediated signaling in HCC with respect to the signaling pathways involved and their physiologic function is unknown. Here we have accessed the role of Wnt11, a member of the noncanonical cascade, in hepatic oncogenesis.
Methods: The expression level of Wnt11 in HCC tumors and adjacent uninvolved liver tissues were determined by real-time RT-PCR. Wnt11 induced effects were accessed by immunofluorescence microscopy, kinase activity and Westernblotting.
Results: over-expression of Wnt11 activated PKC and RhoA activity. PKC promoted phosphorylation of b-catenin, which resulted in reduced intracellular levels followed by inhibition of TCF mediated transcriptional activity and cell proliferation. More important, the expression of Wnt11 mRNA was downregulated in human HCC tumors compared to the adjacent uninvolved liver.
Conclusion: Our observations suggest that Wnt11 antagonizes the canonical pathway through PKC signaling, and functionally inhibits tumor cell growth. Therefore, Wnt11 mediated signaling may play a role as a tumor suppressor and loss of activity contributes to hepatocarcinogenesis.

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A54

IDENTIFICATION OF DISTINCTIVE TCF-4 ISOFORMS THAT EXHIBIT REPRESSIVE FUNCTIONAL CHARACTERISTICS IN HEPATOCELLULAR CARCINOMA

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Background: One of the signaling pathways that lead to hepatocellular carcinoma (HCC) is the Wnt/β-catenin/TCF signaling cascade. During active Wnt signaling, β-catenin is stabilized in the cytoplasm, translocates into the nucleus and activates Wnt target genes by binding to T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors. TCF-4 is dominantly expressed member in HCC. Due to its multiple alternatively spliced exons and splice sites, a specific TCF-4 isoform may dictate the expression of unique Wnt target genes. Therefore, the objective of this study is to identify different TCF-4 isoforms and distinguish their functional effects on HCC cell lines.
Methods: The TCF-4 isoforms were identified by RT-PCR from cDNAs of 4 HCC cell lines. For functional effects, the rate of transcriptional activity and the impact on cell proliferation, apoptosis, cell motility, and cell transformation was analyzed by both transient and stable transfections of each isoform.
Results: We have identified and cloned 14 distinct TCF-4 isoforms. Surprisingly, depending on the presence or absence of specific alternative exons, many of the isoforms showed low TCF transcriptional activity compared to empty vector, yet with different but interesting cell proliferation, migration, and transformation profile.
Conclusion: Our study shows that at least fourteen different TCF-4 isoforms are present in HCC and that not only TCF-4 activates target genes in the presence of β-catenin but also dinstinctive isoforms may inhibit their expression by exhibiting low transcriptional activity.

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A55

TEMPORALLY CONTROLLED OVEREXPRESSION OF CARDIAC-SPECIFIC PI3Kα- A NOVEL TRANSGENIC ANIMAL MODEL

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Problem: The cardiac PI3K signaling pathway is highly regulated during development with the highest levels found during the fetal-neonatal transition period and the lowest levels in the adult. In animal models, constitutively activated overexpression of PI3Kα results in cardiac hypertrophy. Our goal was to demonstrate the phenotype of temporal overexpression of cardiac-specific PI3Kα.
Methods: We have engineered a conditional transgenic mouse line which applies a binary tetracycline (tet) transactivator (tTA) tet-off system to control the overexpression of cardiac-specific PI3Kα. Adult transgenic mice were allowed to overexpress PI3Kα for two weeks and basal myocardial functions were assessed with the Langendorff perfusion system. PI3K activity was measured using *in vitro* lipid kinase assay and protein levels were measured with Western blotting.
Results: There were significantly higher cardiac PI3K activity and Akt activation in PI3Kα- overexpressing mice, compared to control littermates. PI3Kα- overexpressing mice also had significantly increased LV developed pressure, LV dP/dt max and LV dP/dt min. Heart rate and heart/body weight ratio were not changed, suggesting that there is an enhancement in contractility without hypertrophy. Expression of multiple calcium-regulating proteins including L-type voltage-gated calcium channel, RyR receptors and SERCA2a were also increased.
Conclusions: Cardiac PI3K signaling can be conditionally overexpressed in postnatal stages when there are normally very limited PI3K activities, without inducing hypertrophy or cardiomyopathy. Animals overexpressing cardiac PI3Kα have increased contractility with concomitant increases in multiple calcium-regulating proteins. These data suggest there is a fundamental difference in cardiac phenotype between prolonged and temporally controlled overexpression of cardiac PI3Kα.

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A56

DEVELOPMENT OF A GMP FACILITY FOR CELL THERAPY OF CHRONIC NON-HEALING WOUNDS

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With increasing emphasis on translational bedside research, the need for appropriate regulatory oversight and approval has become essential. The requirements of the Food and Drug Administration (FDA) for investigational new drug (IND) studies that are investigators-initiated have become increasingly stringent. We outline the steps that are required for developing an IND submission to the FDA. A substantial obstacle is the development of Good Manufacturing Practice (GMP) facilities. This requires the establishment of Standard Operating Procedures (SOP's) for each piece of equipment, procedures, personnel training and documentation. The GMP facility needs to be approved and is subject to inspection and auditing at any time. There is a strongly intertwined relationship between the GMP, the clinical research team, the Institutional Review Board (IRB), and FDA regulations. For the purpose of illustrating these challenges and how to overcome them, we will take the example of culturing autologous bone marrow-derived mesenchymal stem cells for topical application to non-healing human wounds. In this work, we have shown that cells delivered at a concentration of greater than 1.5 million per centimeter squared of wound surface lead to accelerated healing of previously non-healing wounds. The cells are delivered in a modified fibrin spray construct. All components of treatment, including cells, media, fibrin are subject to SOP and regulatory issues. The development of the GMP facility for this work and the subsequent IND submission to the FDA is a formidable task which academic investigators are not yet familiar with. We will discuss how this can be accomplished.

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A57

EXPRESSION AND ACTIVATION OF MATRIX METALLOPROTEINASES IN THE EASTERN OYSTER *Crassostrea virginica* IN RESPONSE TO EXPERIMENTAL INFECTIONS WITH *Perkinsus marinus*

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Problem: Matrix metalloproteinases (MMPs) are a large family of inducible zinc-dependent proteases capable of degrading components of the extracellular matrix, but also play roles in immune-related functions such as cell migration and inflammation. Previous work in our laboratory has identified an MMP in the Eastern oyster, and the gene for this protease, named Cv1MMP, has been cloned and sequenced. The aim of this work is to investigate the expression and activity of Cv1MMP following 24 hours of experimental infection with *P. marinus*, a protozoan parasite that causes a wasting disease in oysters.
Methods: Oysters were injected with live, seawater activated *P. marinus* and infection intensity was determined using the body burden technique as described by Fisher and Oliver (1996). Serum and hemocyte samples were taken from each individual. Protein concentration was measured using the Better Bradford Assay and bovine serum albumin as standards. Polyclonal antibodies against Cv1MMP were produced by Abgent and used for Western blot analysis.
Results: Western blot detected a band of 38kDa in serum of infected oysters, which was upregulated in response to infection. In addition, the levels of this band are negatively correlated with levels of infection. Immunohistochemical analysis showed that Cv1MMP production is localized mainly in hemocytes and in the lining of the mantle.
Conclusions: This work demonstrates that there is potential for Cv1MMP to act as an immune molecule. The identification and characterization of this matrix metalloproteinase in oysters will aid to our understanding of the specific roles of MMPs in innate immunity.

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A58

ARYLIMINE GSK4716 MIMICS CAN BE SYNTHESISZED THEN MODIFIED BY PHASE-TRANSFER CATALYSIS TO PREPARE ESTROGEN RECEPTOR MODULATORS FOR SCREENING ASSAYS.

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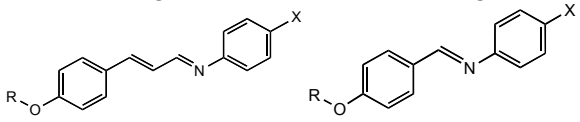
One-step microscale synthesis to prepare a library of arylimines that are structural analogs of GSK4716, a potent selective estrogen receptor modulator (SERM), is underway. After characterization by MP, NMR, IR and GC-MS, these compounds are being screened for estrogen-receptor activity as either agonists or antagonists. Modification of the compounds where X = OH can be done in a subsequent step using a Williamson ether synthesis in a phase-transfer reaction with Aliquat and potassium carbonate. RP-HPLC retention times show good correlations with cLogP values. Virtual docking to the estrogen receptor is underway.



Target Compounds; Variables; R and X

A59

TAMOXIFEN ANALOGS CAN BE SYNTHESIZED BY TRADITIONAL AND PHASE-TRANSFER WITTIG REACTIONS.

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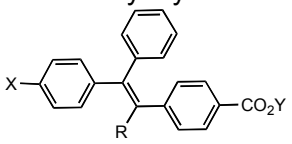
Tamoxifen is a selective estrogen receptor modulator (SERM) and an important treatment and prevention of breast cancer. Unfortunately, this particular drug can cause a higher incidence of other cancers, as well as other complications. We have done proof of principle syntheses opening pathways to yield Tamoxifen-like molecules by both solution phase and phase-transfer Wittig reactions. Compounds from these reactions will be derivatized to create a library of compounds for screening against human breast cancer cell lines and Hep2G cells, the estrogen receptors and the aryl hydrocarbon receptors.



Target Molecule; Variables; X, R, Y

A60

In Silico Screening Protocol for Design of Selective Estrogen Receptor Modulators

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Selective estrogen receptor modulators (SERMs) simulate estrogen activity in the body in certain tissues while having effects distinctly different from estrogen in other tissues. SERMs which inhibit only undesired estrogen activities implicated in carcinogenesis have obvious pharmaceutical value. Using an *in silico* rational drug design workflow employing only open source and/or free for academic use software, triazoles and imines are examined to identify potential SERMs. Combinatorial molecular library design, screening based on "druglikeliness" measures, screening against a neural network trained with known estrogen receptor ligands, virtual docking protocols, and statistical modeling techniques have been used to identify compounds that are predicted to have high binding affinity for the ligand-binding pockets in estrogen receptors alpha and beta as well as estrogen-related receptors alpha and gamma. Furthermore, lead compounds are identified which have the potential for inhibiting specific estrogen receptors and not others.

A61

QSAR-BASED MODEL OF STILBENE DERIVATIVES PREDICTS ESTROGEN ACTIVITY.

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Tamoxifen is a selective estrogen receptor modulator (SERM) employed as
treatment for breast cancer and reducing the incidence of breast cancer inhigh-risk women. The E stereoisomer isomer of a triphenylethylene “Tamoxifen” has very little affinity for its target protein, the estrogen receptor. Once it is metabolized in the liver into the active metabolites 4-hydroxytamoxifen and des-N-methyl-4-hydroxytamoxifen
(endoxifen), these compounds compete with estrogen in the body for binding to the estrogen receptor. Sanoh et al. (2003) have demonstrated that some stilbenes and their related compounds are converted to active estrogen modulators by liver microsomal enzymes. We have calculated QSAR values for compounds examined in this study and constructed a model for predicting estrogen-receptor activity. With this model, QSAR values for a variety of additional stilbenes and similar structures, including Tamoxifen and its derivatives allow predictions about these compounds' activities.

A62

β-ADRENERGIC RECEPTOR MEDIATED PROTECTION AGAINST DOXORUBICIN-INDUCED APOPTOSIS IN CARDIOMYOCYTES: THE IMPACT OF HIGH AMBIENT GLUCOSE

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Problem: Recent studies have demonstrated that the β_2-adrenergic receptor (β_2AR)–$G\alpha_i$ signaling pathway exerts a cardiac anti-apoptotic effect. The goals of this study were to determine the intracellular signaling factors involved in β_2AR-mediated protection against doxorubicin-induced apoptosis in H9c2 cardiomyocyte and to explore the impact of high ambient glucose on the anti-apoptotic effect.
Results: Under physiological glucose environment (100mg/dL), β_2AR stimulation prevented doxorubicin-induced apoptosis which was attenuated by co-treatment with wortmannin, a PI3K inhibitor, or by transfection of a dominant negative Akt. Inhibition of Src kinase with PP2 or cSrc siRNA also attenuated the anti-apoptotic effect. Inhibition of PDGFR with AG1296 reversed the β_2AR-induced anti-apoptotic effect. Transfection of an active Src cDNA (Y529F) alone was sufficient to render the cells resistant to apoptosis and the resistance was blocked by wortmannin. Transfection of an active PI3K minigene (iSH2-p110) alone also induced resistance to apoptosis and the resistance was reversed by an Akt-inhibitor but not by AG1296. High ambient glucose (450mg/dL) caused two major effects: 1) it significantly reduced βAR-induced PDGFR phosphorylation, Src kinase activity and activation of PI3K signaling pathway; 2) it partially attenuated β_2AR-induced anti-apoptotic effect.
Conclusions: These data provide *in vitro* evidence supporting a signaling cascade by which β_2AR exerts a protective effects against doxorubicin-induced apoptosis via sequential involvement of $G\alpha_i$, $G\beta\gamma$, Src, PDGFR, PI3K and Akt. High ambient glucose significantly reduces β_2AR-mediated cardioprotection by suppressing factors involved in this cascade including PDGFR, Src and PI3K/Akt.

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A63

RGS2 IS A NEGATIVE REGULATIVE REGULATOR OF $G_{q/11}$-MEDIATED SIGNALING AND FUNCTION IN ADULT RAT VENTRICULAR FIBROBLASTS

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Cardiac fibroblasts (CF) contribute to cardiac remodeling by assuming an "activated" myofibroblast (MyoFb) phenotype. Many G protein-coupled receptors are involved in CF activation. G proteins are controlled by Regulators of G protein Signaling (RGS). RGS expression and function in CF and MyoFb are unknown. We set out to identify which RGS are expressed in isolated adult rat ventricular CF (passage P0-P2) and to begin to delineate their function. Morphologic changes and expression of MyoFb markers (e.g., α-smooth muscle actin) indicated gradual conversion from CF (P0) to MyoFb (P2). We detected mRNA of 14 RGS by RT-PCR that were differentially regulated during CF to MyoFb conversion. Only RGS2 mRNA was markedly up-regulated in response to short-term stimulation of $G_{q/11}$-coupled Angiotensin II (Ang II) and G_s-coupled β-adrenergic receptors. Adenoviral RGS2 expression blunted Ang II-induced phospholipase C β activation by 65±2% (P0), 66±2% (P1) and 78±3% (P2) (n=3, $P<0.05$), CF proliferation (BrdU incorporation) by 37±5% (n=10, $P<0.05$) and collagen synthesis by 49±11% (n=3, $P<0.05$). RGS2 had no effect on G_s-coupled cAMP accumulation. Thus, while RGS2 is selectively up-regulated in response to $G_{q/11}$ and G_s activation, it negatively regulates only $G_{q/11}$-mediated signaling, proliferation and collagen synthesis in CF and MyoFb. These data point to RGS2 as an important regulator of CF and MyoFb signaling and function.

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A64

Inhibition Of Histone Deacetylases Triggers Delayed Pharmacologic Preconditioning Effects Against Myocardial Ischemic Injury

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Problem: It has been demonstrated that inhibition of histone deacetylases (HDAC) blunts cardiac hypertrophic response. It remains unknown about the role of HDACs inhibition in myocardial ischemia and reperfusion injury. Our goal was to investigate: 1) whether HDACs inhibition triggers delayed cardioprotection against myocardial ischemic injury and 2) whether cardioprotection elicited by HDACs inhibition is associated with p38.
Methods: Mice were treated with either trichostatin A (TSA) (0.1mg/kg, i.p.), a potent inhibitor of HDACs or saline. Twenty four hours later, the hearts were perfused in the Langendorff mode and subjected to 30 min of ischemia followed by 30 min of reperfusion. Left ventricular function was measured and infarct size was determined by triphenyltetrazolium chloride. Western blot was used to detect HDACs 3, 4, and 5 proteins and p38 phosphorylation.
Results: TSA produced marked improvements in the recovery of left ventricular end-diastolic pressure (LVEDP), LV-dP/dt max, and LV-dP/dt min and rate pressure product. TSA-induced improvements were completely abrogated by SB203580, a specific inhibitor of p38. TSA treatment led to reduction in myocardial infarct size, which was also blocked by p38 inhibition. Western blot showed abundant HDAC 3, 4 and 5. TSA treatment resulted in significant inhibition of HDAC activity in preconditioned hearts as compared to the vehicle group. Additionally, HDACs inhibition caused a dramatic increase in phosphorylation of p38. Immunoprecipitation assay revealed that HDACs inhibition resulted in p38 acetylation.
Conclusion: These results suggest that the direct inhibition of HDACs triggers pharmacologic preconditioning, which is dependent on signaling mechanisms involving p38 activation.

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